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Individual variations in ability to acclimatize to high altitude

BY ANCEL KEYS, BRYAN H. C. MATTHEWS, W. H. FORBES AND ROSS A. McFARLAND

From the Fatigue Laboratory, Harvard University, Boston, Massachusetts, U.S.A., the Physiological Laboratory, Cambridge, and the Psychology Department, Columbia University, New York, U.S.A.

(*Communicated by Sir Joseph Barcroft, F.R.S.—Received 27 April 1938*)

WITH A SUPPLEMENT BY R. A. FISHER, F.R.S.

PART I. THE PREDICTION, FROM PHYSIOLOGICAL CHARACTERISTICS AT SEA-LEVEL, OF THE ABILITY TO ACCLIMATIZE

A striking feature in the acclimatization of man to high altitude is the great variability in the responses of different individuals to the lowered air pressure. This has been observed repeatedly by mountaineers and was very evident during the work of the International High Altitude Expedition to Chile in 1935. Our experience was in agreement with other reports: (1) individuals of similar age and general physical condition differ widely in their ability to adjust to high altitude, (2) the differences between individuals become increasingly marked at successively higher altitudes, (3) the initial response to the high altitude is not always a good indication of the eventual acclimatization to be reached, (4) good acclimatization to a moderately high altitude is not necessarily an indication that relatively good acclimatization will be attained at very high altitudes.

It is both theoretically and practically of importance to attempt to evaluate some of the factors involved in making for the variation of individuals in the ability to acclimatize to high altitude. For example, it would be of both practical and theoretical value to be able to predict, in a given group of men, which individuals would be most likely to acclimatize well to high altitude and which would probably do poorly. A considerable measure of success has been attained by aviation medical boards and committees in the selection of candidates for high flying, but the problems involved in *acclimatization* to high altitude are very different.

The present paper is an attempt to relate some of the physiological characteristics of the members of the Expedition to the relative degrees of acclimatization attained. Three main questions will be considered: (1) the prediction of relative ability to acclimatize from observations at sea-level, (2) the relation between the degree of acclimatization and the physiological characteristics of the individual at high altitude, and (3) the relation between subjective and objective judgment of acclimatization.

High-altitude stations

Five high altitude stations were established during a period of four months in the field, Table I gives the altitudes and periods of occupation. In the field the members of the Expedition lived and worked together under conditions permitting close observation of each other. At Chuquicamata (2.81 km.) and Collahuasi (4.70 km.) the men lived in a Pullman-type railroad car, whilst at 'Quilcha (5.34 km.) a well-built house was occupied. At Punta (6.14 km.) the living quarters consisted of two small "glacier" tents and a snow cavern. Except at the highest station, living conditions were sufficiently normal and comfortable so that environmental abnormalities extraneous to the high altitude itself were largely avoided.

TABLE I

Station	Period of occupancy	Corrected barometric pressure	Altitude Metres	Altitude Feet
Chuquicamata	8 April to 4 June	543	2,810	9,200
Ollague	5 to 13 June and 25 June to 18 July	489	3,660	12,020
Collahuasi (Montt)	13 to 25 June	429	4,700	15,440
Aucanquilcha	26 June to 15 July	401	5,340	17,500
Punta de Cerro	29 June to 14 July	356	6,140	20,140

The ten members of the Expedition were all men in good health, of fairly active habits, and were all considered to be good "risks" to undertake a prolonged stay at high altitude. At the various stations the time was occupied with experimental and analytical work, with occasional holidays walking and climbing (for a general description of the stay of the Expedition in the field, see Keys 1936).

The relation between acclimatization and physiological characteristics

It is unfortunately true that, as yet, there is no certain knowledge as to precisely what constitutes acclimatization to high altitudes. The work of the

International High Altitude Expedition has, however, cleared much ground in that some of the most widely championed theories have been eliminated Acclimatization to high altitude apparently does *not* consist in the development of oxygen 'secretion" by the lungs (Dill, Christensen and Edwards 1936), *nor* does a shift in the oxygen dissociation curve of the blood or haemoglobin explain the adjustment* (Keys, Hall and Barron 1936)

Judgement of relative acclimatization

Each member of the Expedition independently judged the relative acclimatization of all the Expedition members including himself, by classifying the ten men in groups as follows (1) the three men relatively best acclimatized, (2) the three men least well acclimatized, and (3) the four intermediate men These judgements were made for each high altitude station except that stations 'Quilcha and Punta (5 34 and 6 14 km) were combined in view of the lack of opportunity for mutual observation at Punta †

To provide a numerical basis for analysis, points were assigned as follows (1) best group—10 points each, (2) intermediate group—5 points each, (3) poorest group—0 point Table II shows the summarized ratings of the members of the Expedition ‡

At the outset it will be admitted that these ratings are only relatively accurate at best Extraneous psychological factors are involved to some extent and must have affected both the responses of the individuals to the high altitude—and therefore their apparent acclimatization—as well as the judgements themselves Moreover, the assignment of numbers to the successive rankings of acclimatization injects an arbitrary element into the measure of acclimatization for which it is difficult to make a precise allowance, particularly in studies of correlation However, we shall be concerned only with general and relative conclusions, and it should be noted, also, that all the Expedition members were experienced observers and that objective bases for judgement were constantly afforded in the relative readiness and ability of the men to take part in experiments, analytical work and diversions

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* It is true however, that slight and transient changes in the position of the oxygen dissociation curve, perhaps associated with maladjustment, may be found at high altitude

† Only six men, B, F, K, Mc, M, and P actually camped at 6 14 km , and only F, K, and M stayed there more than 2 nights

‡ Six members of the Expedition submitted complete ratings on a scale of 1 to 10 for the relative acclimatization at each station The mean result of these estimates agrees closely with the mean estimates obtained by grouping

In addition to the group judgements of relative acclimatization there were three other bases for assigning acclimatization ratings to the several members of the Expedition (1) self-judgements in which each individual rated his own relative acclimatization, (2) the results of questionnaires on physiological "complaints" which were filled in by each member for each of the high-altitude stations, (3) the results of questionnaires on psychological "complaints" filled in by each member for each of the high-altitude stations These are discussed later in this paper, but ratings made by all of these methods agreed remarkably well with the group judgement ratings

TABLE II SUM OF TEN SEPARATE JUDGEMENTS OF RELATIVE DEGREE OF ACCLIMATIZATION AT THE HIGH-ALTITUDE STATIONS BASED ON A SCORE OF 10 POINTS FOR INCLUSION IN THE GROUP OF THREE MEN BEST ACCLIMATIZED, 0 FOR THE THREE MEN LEAST WELL ACCLIMATIZED, AND 5 POINTS FOR INCLUSION IN THE GROUP OF FOUR MEN IN THE INTERMEDIATE CLASS MAXIMUM POSSIBLE SCORE 100, MINIMUM 0

Name	2 81 km	3 66 km	4 71 km	5 34 and 6 14 km	All stations	Max variation between any two stations
B	39	60	70	65	59	31
C	65	50	10	5	32	55
D	14	55	35	33	34	41
E	35	35	50	62	46	27
F	49	70	65	75	65	36
H	30	0	5	5	10	30
K	100	95	85	85	91	15
Mc	25	0	20	58	26	58
M	90	80	87	90	87	20
T	54	55	73	17	50	56

Table II shows that there is a general tendency at different altitudes for the various men to maintain their general positions with respect to relative acclimatization, but that there are exceptions This is marked in the cases of C and T* who deteriorated at the highest stations and of Mc who improved considerably The mean scores for all stations, however, are probably not far from correct except for Mc, who may be placed lower than his physical acclimatization would warrant †

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* The deterioration of T may have been overestimated, his very severe mountain sickness at Punta (6 14 km) impressed us all, but, as mentioned before, not all of the party underwent the test of camping at Punta

† Mc suffered from a cold and sore throat at the lower stations, and this may have unduly influenced the judgement of him though we attempted to allow for this

Sea-level characteristics and high altitude acclimatization

The principal physiological characteristics of the members of the Expedition at sea-level are given in Table III For the purpose of exploring possible methods for the prediction of acclimatization from observations at sea-level we may consider the following characteristics of the individual at sea-level

(1) *Age* It is commonly observed that the ability to adjust to new conditions of all kinds is usually related, very roughly at least, with age There is some reason from mountaineering experience to think this may also be true of acclimatization to high altitude

(2) *Body weight* Mountaineers have frequently suggested that there may be a relation between body size and ability to acclimatize to high altitude

(3) *Pulse rate* It is conceivable that the basal pulse rate of normal men at sea level may reflect the efficiency of the circulation at sea-level and the reserve which may be called on to meet the more difficult requirements at high altitude

(4) *The alveolar pressure of oxygen* A low alveolar pO_2 will mean a low ventilation relative to the oxygen consumption and this may imply an economy of respiration

(5) *The alveolar pressure of carbon dioxide* We may apply the same argument (inversely) to the pCO_2 as to the pO_2

(6) *The alkaline reserve* The amount of CO_2 which blood will hold at $pCO_2 = 40$ mm is related to the respiratory habit of the individual As such it may supply information similar to that of the alveolar pCO_2 except that it should reflect the respiratory history of some hours or days

(7) *Oxygen capacity of the blood* A high oxygen capacity would at first sight seem to be a favourable factor for eventual adjustment to low oxygen conditions However, it would seem more reasonable to argue that if a high O_2 capacity is needed for sea level conditions this may mean an inefficient use of that haemoglobin

(8) *Schneider s Index* This value is obtained from values of the basal pulse, standing pulse, pulse following a brief standard exercise, and blood-pressure values It purports to be a measure of neuro-circulatory fitness (cf Schneider 1933)

Some other characteristics which might be suggested as of use in this connexion may be excluded on one of two grounds (1) Normal variations at sea-level are practically within the limits of measurement (arterial O_2 saturation, arterial pH, the position of the oxygen dissociation curve etc), (2) the values are largely dependent on the variables considered above (arterial partial pressure of oxygen, erythrocyte count, cell volume per cent, etc)

Finally, it might be suggested that some functional test, e g the response to a fixed muscular task, would be useful Aside from the difficulty of fixing on a reliable and reproducible task and measurements of the responses, it is generally agreed that simple muscular power at sea-level is a very poor indication of relative ability to acclimatize to high altitude This was further emphasized during the stay in the field of our Expedition

The correlations between each of the variables studied and the mean acclimatization subsequently observed at high altitude are given, together with the correlation coefficients, in figs 1–8 * The arterial pH's and percentage O_2 saturation were not so treated because their variations are practically within their experimental and analytical errors General physical fitness and past athletic activity are probably of some importance but it is not easy to evaluate these characteristics in any quantitative manner Moreover, one may expect that at least some of the effects of general fitness and previous athletic history should be reflected in the other characteristics measured

The relations between the separate physiological characteristics at sea-level and the observed acclimatization in the ten Expedition members are shown in figs 1–8 There are indications of rough but well-marked correlations between ability to acclimatize and the sea level characteristics as follows direct—pCO_2 and alk res , inverse —age, pulse, pO_2, O_2 cap , and wt † Only the Schneider Index shows no marked correlation, this may be owing to the very small range of values for the S Index in our party

Inverse relations between acclimatization and age and pulse rate might have been expected The general direction of the relations with alveolar tensions likewise seems reasonable, a low pO_2 and high pCO_2 at sea-level should mean, other things being equal, an economical and efficient respiratory mechanism ‡ A high alk res may be taken to be related to habitual

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* It is realized that the correlation coefficient is difficult to interpret as a quantitative measure, particularly so since one of the variables (acclimatization) is not an actual measurement but a numerical scoring from relative ranking However, since the acclimatization measures are the same for all the figures, we feel that the correlation coefficient can be used to gauge the relative importance of the sea level characters as factors in determining the ability to acclimatize

† The following abbreviations are used throughout this paper Oxygen capacity of haemoglobin (c c HbO_2 per 100 c c blood) = O_2 cap , partial pressures of oxygen and carbon dioxide in the alveolar air = pO_2 and pCO_2 respectively, alkaline reserve of the oxygenated blood (c c CO_2 contained when the CO_2 pressure is 40 mm) = alk res , Schneider Index = S Index, basal pulse rate = pulse, body weight = wt

‡ It will be noted that the arterial saturation with oxygen at sea level was almost constant in all of the men (Dill, Christensen and Edwards 1936) Likewise, the position of the oxygen dissociation curve at sea level was relatively constant (Keys, Hall and Barron 1936) A high pO_2 value in the alveolar air, then, must mean a relatively poor mechanism for the uptake of oxygen in the lung

TABLE III CHARACTERISTICS OF THE EXPEDITION MEMBERS AT SEA-LEVEL (IN BASAL REST) BEFORE ASCENT AND RELATIVE ACCLIMATIZATION TO HIGH ALTITUDE AS ASSESSED BY VARIOUS METHODS

	Relative acclimatization											Arterial blood					
												Alv air					
Name	Group judging	Self judging	Physiological questions	Psychological questions	Age	Ht cm	Wt kg	Basal pulse rate	Fitness	Athletic history	Schneider Index	pO_2	pCO_2	O_2 cap vol %	% O_2 sat	pH's	T_{40}
K	91	100	88	75	31	171	68.1	51	Good	Mod	13	96	44	18.6	95.7	7.39	49.6
M	87	100	80	78	29	184	76.2	59	Good	Much	12	100	43	19.6	—	—	—
F	65	50	70	75	33	176	69.4	52	Good	Mod	11	101	43	20.4	95.8	7.43	49.9
B	59	74	62	70	37	161	60.8	54	Fair	Little	14	97	43	19.4	95.3	7.36	46.6
T	50	63	—	—	34	169	74.8	61	Good	Mod	13	102	39	20.7	95.6	7.39	48.7
E	46	74	28	12	38	166	71.2	64	Fair	Little	13	99	41	19.6	96.8	7.38	47.7
D	34	37	35	40	44	177	77.1	56	Good	Much	12	103	41	18.2	95.1	7.38	49.4
C	32	37	45	30	30	177	75.5	62	Good	Much	14	111	36	19.3	95.2	7.40	48.1
Mc	26	50	32	28	33	191	86.2	62	Good	Much	13	103	42	20.9	95.2	7.38	48.3
H	10	12	12	38	39	173	72.6	61	Fair	Little	13	103	39	22.1	94.6	7.39	45.3
Mean	50	60	50	50	34.8	174.5	73.2	58.2			12.8	101.4	41.1	19.9	95.5	7.39	48.2

Pulse rate Mean of determinations on about 10 different days

O_2 cap Mean of determinations on 2–5 different days

Alv pO_2 and CO_2 Mean of determinations on 4–15 different days

$T_{40} = CO_2$ content, in c c per 100 c c of oxygenated whole blood at 38° C when $pCO_2 = 40.00$ mm Hg pO_2 and pCO_2 are in mm Hg high pCO_2 and might, therefore, be expected to show a somewhat similar relation

At first sight it may seem unreasonable to suggest that a high O_2 cap of the blood at sea-level should prognosticate poor acclimatization to high altitude However, among healthy well-nourished people there is little reason to suspect that variations in the O_2 cap within the normal range bear

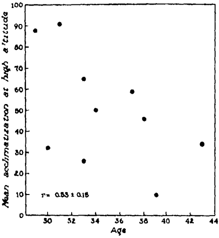

FIG 1

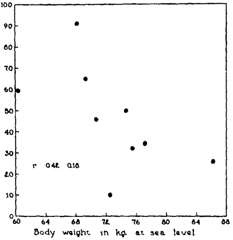

FIG 2

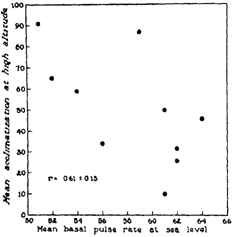

FIG 3

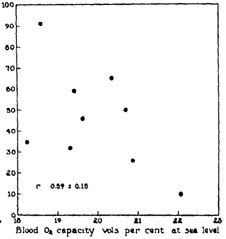

FIG 4

FIGS 1–4 Relation between observed mean acclimatization to high altitude (2800–6140 m) and physiological characteristics measured in basal rest at sea level before going to high altitude For normal men between the ages of 29 and 44 habituated to life at sea level any direct relation to physical vigour or physiological responsiveness It is a common opinion, based on little or no evidence, that athletes have higher O_2 caps than non-athletes, but this is not borne out by the meagre data available Moreover, there is no relation, amongst athletes, between athletic ability and the O_2 cap Amongst non-athletes, such as the members of the Expedition, it can be argued that a high O_2 cap should give a poor prognosis,

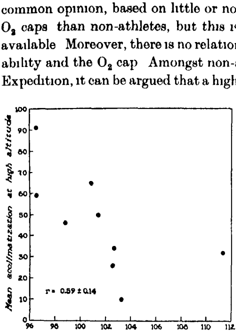

FIG 5

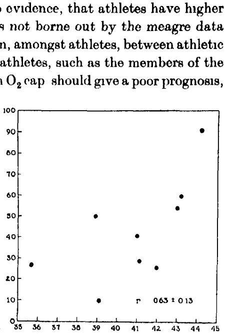

FIG 6

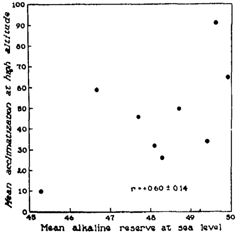

FIG 7

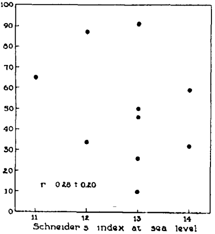

FIG 8

FIGS 5–8 Relation between observed mean acclimatization to high altitude (2800–6140 m) and physiological characteristics measured in basal rest at sea level before going to high altitude For normal men between the ages of 29 and 44 habituated to life at sea level i e if an unusually high O_2 cap is needed to meet the ordinary exertions at sea-level, what can be expected at high altitude? We may recall the fact that in chronic conditions where the respiratory-circulatory mechanism is inefficient, as in cardiac disease, the O_2 capacity is elevated more or less in proportion to the severity of the condition

We take it that acclimatization to high altitude depends on the combined action of a number of physiological factors If we may assume some kind of summation of effects, then examination of the relations between acclimatization and combinations of the characteristics plotted singly in figs 1–7 (omitting Schneider's Index which shows no correlation) should show whether we are correct in our suggestion that these characteristics are definitely related to some of the factors involved in acclimatization

There are several possible methods by which the variables might be combined for this purpose A rigorous approach from the standpoint of pure statistics was not available to use at the time this paper was written, in the supplement to this paper Professor R A Fisher has developed such a method

Our first method was purely empirical and consisted of the construction of correlation charts for the observed acclimatization with each of the 120 possible combinations of the seven variables The combinations were made by algebraic summation, taking account of the sign of the individual correlations Thus we used, e g , age + pulse, age + pO_2 − alk res , etc Since there was no reason to suspect that any one of the variables is more important than the others for the present purpose, it was decided to give them all equal weight Numerically this means that the units in which the several variables are expressed should be so chosen that the range of variation for all of the variables was relatively constant More precisely, we chose the units for each characteristic so as to make the standard deviations the same for all

Space does not permit reproduction of the 120 correlation charts here It was found almost without exception that the combinations of two characteristics yielded higher correlations with acclimatization than any single characteristic, and that on increasing the number of characteristics combined, the correlations were improved still further

The graphical analyses appear to justify our suggestions, viz (1) The sea-level characteristics considered in this paper—age, pulse, pO_2, pCO_2, O_2 cap , alk res , and wt —are related to some, if not all, of the factors which determine the ability to acclimatize to high altitude (2) In general, the direction of these relations is compatible with physiological theory (3) Algebraic summation of the sea-level characteristics gives a closer estimate of the ability to acclimatize than does any one of the separate characteristics and, in general, the more characteristics are combined, the better is the estimate

The success attained, as judged by the improvement of the correlation with acclimatization, with this simple method of combining physiological characteristics suggested that it would be worth while to attempt to combine the variables in the best possible equation by the method of the least squares This was done and the results are given in fig 9

The extraordinarily good correlation shown in fig 9 is not to be interpreted to mean that the predictive value of our equation in seven variables is

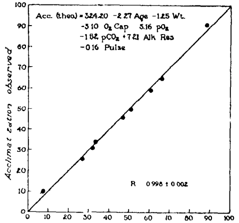

FIG 9 The prediction of acclimatization to high altitude from the best combination of physiological characteristics measured at sea level before going to high altitude Best combination obtained by method of least squares

equally good As shown in the supplement to this paper a high degree of correlation may be expected in a system, like the present, in which the number of variables approaches the number of individuals Fig 24 is a correlation chart in which only four variables—age, body weight, alkaline reserve and alveolar pO_2—have been used by Professor R A Fisher to provide an equation of high predictive value

These results would seem to mean that, in our group, we measured physiological characteristics at sea-level which, directly or indirectly, included the more important factors involved in the determination of the eventual acclimatization of the individual at high altitude as judged by our method of observation It is not pretended that our findings will apply precisely to other groups It does seem reasonable to conclude, however, that the general character and direction of the relations should hold when applied to groups of men reasonably comparable to our own By "reasonably comparable" we may suggest relatively normal men, psychologically stable, habituated to sea-level between the age limits of 28 and 45

The best test of the general applicability of these concepts would be to analyse the results of other high altitude expeditions in similar fashion Unfortunately this is not possible, previous expeditions did not collect the requisite data In the case of the Anglo American Expedition to Peru (Barcroft and others 1923), however, a rough classification of the eight members in respect to acclimatization is possible * The four men who were judged to acclimatize best averaged 34 years of age at the time of the Expedition, the other four men averaged 38 years of age Such as it is, the relation of acclimatization with age was similar to the present Expedition Obviously, we must await further studies on groups of people going to high altitudes

PART II THE RELATION BETWEEN OBSERVED ACCLIMATIZATION AND PHYSIOLOGICAL CHARACTERISTICS IN REST AT HIGH ALTITUDE

In a group of men going from sea-level to high altitude there is little difficulty in arriving at a general agreement as to the relative degree of acclimatization achieved by the different individuals, provided opportunities are afforded to observe them carefully for some days at work, at meals, at recreation and in exercise At rest, however, such discrimination has not been attempted previously, medical examination has been used only for the evaluation of the seriousness of definite mountain sickness when this is present

This apparent superiority of general observation over brief examination and measurement depends, we think, on lack of serious trial and study of physiological measurements The data collected by the International High Altitude Expedition in Chile in 1935, inadequate though they may be, are far superior to any available previously In this part of the present paper the relation between observed acclimatization and a number of physiological variables at high altitude will be analysed

The acclimatized man at high altitude differs, in physiological characteristics, from the sea level man in a number of ways (1) He has a considerably higher concentration of red cells and a correspondingly higher oxygen

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* The following members of the Anglo American Expedition kindly supplied estimates of the relative acclimatization of the Expedition members Professor Sir Joseph Barcroft, F R S, Professor A V Bock, Dr H S Forbes, Professor G A Harrop, Jr, and Professor Alfred C Redfield

capacity of his blood, and this excess is more or less proportional to the altitude (Talbott 1936, Keys, Hall and Barron 1936) (2) His arterial blood is unsaturated to a degree which is also more or less proportional to the altitude (Barcroft and others 1923, Dill, Christensen and Edwards 1936, Monge and others 1928, Hurtado 1931) (3) His blood shows a reduction in carbon dioxide capacity roughly proportional to the altitude, in spite of this his arterial blood has about the same pH as at sea level in rest (Dill, Talbott and Consolazio 1937) (4) His heart rate in rest is as slow or slower than at sea level, mild exercise increases it abnormally, but the maximal heart rate he can reach in work is less than at sea level (McFarland 1937 Christensen 1937, this paper, below) (5) His respiration rate and pulmonary ventilation are increased, particularly in work, and the difference between the oxygen pressure in the lungs and in the arterial blood is diminished (Barcroft 1925 Haldane and Priestley 1935, Dill and others 1936, Christensen 1937) (6) He exhibits signs of generally increased sensitivity of the vegetative nervous system, with hypertonus of the vagus and sympathetic nerves (Monge and others 1928) (7) He reaches exhaustion in anaerobic work without accumulating anything like as much lactic acid in his blood as at sea level (Edwards 1936) (8) At very high altitudes sensory and motor responses are diminished in intensity or speed and mental operations proceed with more difficulty than at sea-level (McFarland 1937)

The method of evaluating acclimatization and the relative acclimatization scores for the members of the Expedition at different altitudes are given in Part I of this paper Here we shall consider only the mean acclimatization and the mean physiological characteristics at the combined stations Montt (4 70 km) and 'Quilcha (5 34 km) where the measurements were most complete These are given in Table IV

The relations between the separate characteristics at high altitude and the observed acclimatization at the same altitude are shown in figs 10–19 Marked correlations exist between acclimatization and the following characteristics basal pulse (inverse), standing pulse (inverse), the change of pulse rate from basal to standing (inverse), the Schneider Index score (direct), and the position of the oxygen dissociation curve, pO_2 at Hb = HbO_2 at $pH_0 = 7\ 10$ (inverse) Less pronounced correlations exist between acclimatization and oxygen capacity of the blood (inverse), arterial O_2 saturation (direct), alkaline reserve (inverse, questionable), the oxygen pressure gradient in the lung (inverse), alveolar pO_2 minus arterial pO_2 (inverse) and age (inverse)

The inverse relations between pulse rates (basal, standing, and change from basal to standing) and acclimatization are not surprising Mountaineers

TABLE IV MEAN CHARACTERISTICS OF MEMBERS OF THE EXPEDITION AT MONTT (4 70 KM) AND 'QUILCHA (5 34 KM)

Name	Age	Relative acclimatization	Basal pulse	Standing pulse	Schneider s Index score	Arterial % sat	pO_2 at Hb = HbO_2 (pH_e = 7 10)	ΔpO_2, Alv − Art	Alkaline reserve	Alveolar pO_2 mm	O_2 cap , vol %
E S G B	37	68	69	86	9 5	76 6	29 1	+1 0	42 0	43 5	26 7
E H C	30	8	77	102	7 5	79 6	30 3	+5 4	43 0	49 0	24 2
D B D	44	34	62	78	8 5	68 0	30 1	+1 5	42 8	40 9	21 9
H T E	38	56	70	93	6 0	77 0	26 5	+2 6	39 3	42 8	25 0
W H F	33	70	56	77	10 5	81 1	28 8	+3 4	40 8	47 6	23 7
F G H	39	5	69	102	6 0	79 4	29 2	+1 8	39 0	46 9	25 8
A K	31	85	52	65	12 0	81 8	27 3	+0 8	39 8	46 3	23 4
R A Mc	33	39	72	90	8 5	80 5	29 1	+2 5	40 0	49 3	24 2
B H C M	29	89	59	84	8 0	79 4	29 1	+0 4	40 8	45 7	24 1
J H T	34	40	—	—	—	73 2	29 3	−2 7	41 0	39 6	24 2
Mean	34 8	—	65 1	86 3	8 5	77 7	28 9	+1 7	40 9	45 2	24 3

and explorers have frequently commented on the rapid pulse and the marked rise, on standing, in people suffering from mountain sickness In the fully acclimatized miners at 'Quilcha we found very slow pulse rates, the average for the basal pulse in thirty-five miners at 5 34 km was 52 per min It would seem, then, that there is a marked inverse correlation between pulse and acclimatization over the whole range from acute mountain sick-

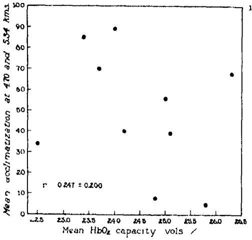

FIG 10

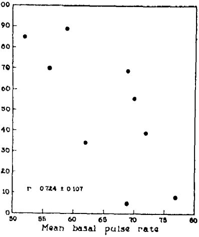

FIG 11

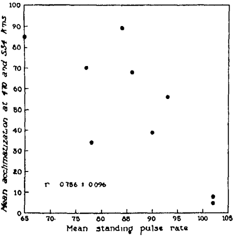

FIG 12

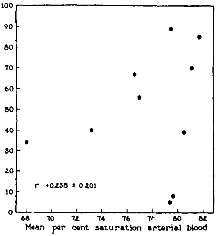

FIG 13

FIGS 10–13 Relation between observed mean acclimatization to high altitude (4700 and 5340 m) and physiological characteristics measured in basal rest at the same altitude For normal men between the ages of 29 and 44, habituated to life at sea level and incompletely adjusted to the high altitude ness to complete acclimatization The Schneider Index score appears to be of less value in this connexion, the value it does possess is owing to the inclusion in it of the reclining and standing pulse rates

The relatively small utility of the arterial oxygen saturation in a measure of acclimatization is explicable in view of the fact that the miners, fully acclimatized to 5 34 km altitude, had the same average arterial oxygen saturation as did our much less well acclimatized group at the same altitude (cf Keys, Hall, and Barron 1936, Dill, Christensen and Edwards 1936) All

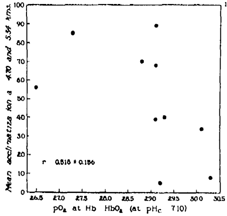

FIG 14

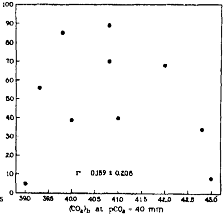

FIG 15

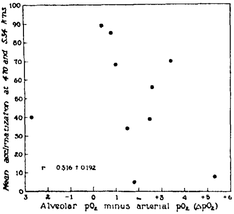

FIG 16

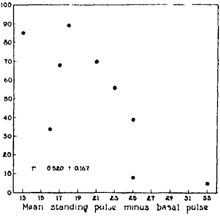

FIG 17

FIGS 14–17 Relation between the observed mean acclimatization to high altitude (4700 and 5340 m) and physiological characteristics measured in basal rest at the same altitude For normal men between the ages of 29 and 44, habituated to life at sea level and incompletely adjusted to the high altitude the facts now point to the conclusion that acclimatization does not consist of, or even involve importantly, any improvement in the saturation of the arterial blood In the initial stages of acclimatization at high altitudes, however, the stimulus to the necessary enhanced respiration may not be developed (e g the blood *p*H may be very alkaline) and more than the degree of anoxaemia characteristic of that altitude may prevail for a time This would account for the slight correlation actually found

The inverse correlation, in our group, between acclimatization and the blood oxygen capacity at high altitude may seem strange in view of the fact

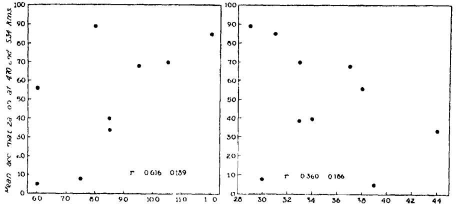

Fig 18

Fig 19

Figs 18, 19 Relation between observed mean acclimatization to high altitude (4700 and 5340 m) and physiological characteristics measured in basal rest at the same altitude For normal men between the ages of 29 and 44 habituated to life at sea level and incompletely adjusted to the high altitude

that the O_2 capacity of the blood from the long-time residents is much higher than that of the blood of the newcomers who are much less well acclimatized However, in the case of newcomers to high altitude the blood O_2 capacity will reflect, for some weeks, the sea-level value of the O_2 capacity, in Part I of this paper we have already indicated how a relatively high O_2 capacity at sea-level may indicate an inefficient respiratory or circulatory mechanism (cf p 10)

The position of the oxygen dissociation curve is of interest The change at high altitude seems to be temporary and the long-time residents do not exhibit it (Keys, Hall and Barron 1936) The magnitude of the change, then, could well represent a kind of measure of incompleteness of acclimatization This is in full agreement with the observed relation between acclimatization and the shift of the oxygen dissociation curve from the sea-level position

Our finding that a large diffusion gradient in the lungs is related to poor acclimatization would be expected from all points of view (cf e g Douglas, Haldane, Henderson and Schneider 1913, Harrop 1922, Dill, Christensen and Edwards 1936) It might have been expected that the relation would be more pronounced than actually found There are two obvious reasons why this is not the case In the first place the measurement of the diffusion gradient is very complicated and difficult so that great accuracy is almost impossible Secondly, at really high altitudes the diffusion gradient becomes so small in all normal men that it is to be doubted that it can be a principal limiting factor in acclimatization, except in special cases In those cases where we measured the diffusion gradient in the long-time residents the values were not significantly different from our own

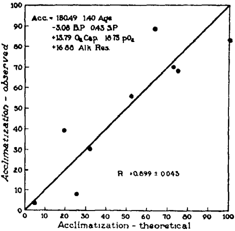

FIG 20 The relation between mean observed acclimatization to high altitude (4700 and 5340 m) and the best combination of physiological characteristics measured at the same altitude Best combination obtained by the method of least squares

Finally, we should have predicted that, in a party like our own, the alkaline reserve at high altitude would not be closely related to the state of acclimatization Under the conditions of our measurements, the alkaline reserve must be determined by the previous value at sea-level, the respiratory history at high altitude including effects resulting from unusual exertions, etc , the diet, and the compensatory adjustments of the kidney In other words the connexion with the acclimatization process is indirect and, in our case, there was insufficient time for final renal readjustments

Summation of the physiological characteristics at high altitude gives, as with the sea-level characteristics, a better correlation with acclimatization than any single characteristic The combination, by the least squares method, of the high altitude characteristics in an equation to give the best correlation with observed acclimatization is shown in fig 20 The coefficient of correlation between the observed and the "theoretical" acclimatization from this equation is + 0 898 ± 0 043 This is a good but not a perfect correlation, apparently our physiological measurements included most but not all of the significant variables

It should be noted that the interpretation of the coefficient of correlation here, as in the case of the combination of sea-level characteristics, must be made with caution We are justified in concluding only that a combination of the variables considered provides a reasonably accurate measure of the relative acclimatization

PART III THE RELATION BETWEEN REPORTED SUBJECTIVE EXPERIENCES AND THE OBJECTIVE JUDGEMENT OF ACCLIMATIZATION

Many investigators have noted that in acute anoxaemia the subject himself is unaware of his general condition and his impaired faculties (cf e g Haldane and Priestley 1935) By analogy, it is frequently assumed that, when true mountain sickness does not intervene, subjective experiences alone offer a poor guide to the judgement of relative acclimatization During the stay in the field of the International High Altitude Expedition several varieties of evidence were collected on this point

In the first place, inspection of the rating lists of relative acclimatization submitted by the members of the Expedition shows that the individual's judgement of himself did not differ greatly, in our party, from the general judgement of his acclimatization Fig 21 *a* shows the correlation between the judgement of the individual by himself and the judgement of his fellows The correlation coefficient of $r = +0\ 901 \pm 0\ 040$ was calculated It will be noted that nine out of ten of the Expedition members judged themselves somewhat more favourably than did their fellows, this may be put down to vanity, or to inability to *feel* the effects of the altitude upon themselves though they could *see* them in others or, more probably, a mixture of these two

Independent evidence was obtained by two questionnaires, one of thirty-five questions concerning physiological complaints and one of thirty questions on psychological complaints The forms were arranged so that each subject could indicate the relative frequency or entire absence of each complaint At each of the high-altitude stations these forms were filled in by the various members of the Expedition in private and these replies were not inspected until after the return to sea-level The questions are reproduced in Table V

The frequency of reported "complaints", as indicated by the replies to the questionnaires, was found to be directly related to the altitude (cf McFarland 1937) Fig 21 *b* shows the relation for both physiological and

TABLE V QUESTIONNAIRE ON SUBJECTIVE COMPLAINTS AND ABNORMALITIES

Physiological

1 Feel poorly rested in the morning
2 Troubled with sleeplessness
3 Frontal headache
4 Easily fatigued on exertion
5 Visual or auditory impairment
6 Vertigo or dizziness
7 Irregular pulse
8 Precordial pain or distress
9 Cold extremities
10 Feeling of heat and sweating
11 Nose bleeding
12 Shortness of breath on slight exertion
13 Periodic or irregular breathing
14 Sighing or long deep breaths
15 Irregular breathing at night
16 Gas in stomach or intestines
17 Nausea or indigestion
18 Vomiting or anorexia
19 Loss of appetite
20 Tremors of fingers, hands, eyelids, etc
21 Diarrhoea
22 Constipation
23 Ringing or buzzing in ears
24 Aching of muscles without exercise
25 Feel stiff and cramped
26 Impaired coordination or clumsiness
27 Tightness across chest
28 Physically depressed and miserable
29 Pains in various parts of body
30 Cyanosis
31 Lassitude
32 Feelings of suffocation at night
33 Frequency of urination
34 Dryness of skin
35 Sore throat

Psychological

1 Difficulty in remembering
2 Slowness in reasoning
3 Easily irritated or upset
4 Greater effort to carry out tasks
5 Difficulty in concentrating
6 Frequently recurring ideas
7 Worry over trifles
8 Feelings easily hurt
9 Diminution of strength of purpose
10 Sudden changes of mood
11 Mind wanders easily—distractible
12 Mentally lazy
13 Feel depressed and grouchy
14 Greater dislike of criticism
15 Fidgety or restless
16 Heightened sensory irritability
17 Dream during night
18 Worry about affairs at home
19 Feel gay and exhilarated
20 Easily upset by noises or interruptions
21 Worry excessively about health
22 Nervous, high strung, inward tension
23 Consciousness of sex
24 Critical of other people
25 Touchy on various subjects
26 Stuttering or blocking of speech
27 Fussy about food and eating
28 Slow in making decisions
29 Dislike being told how to do things
30 Feel discontented and unhappy

Frequency of appearance of the various "complaints" was indicated in the questionnaires used in the field by checking columns headed "often", "seldom", "never" psychological complaints with altitude It should be noted that these "complaints" are supposed to represent subjective sensations and feelings beyond what the several Expedition members considered to be normal for sea-level In fact, however, it is probable that some of these complaints would have been recorded in an equal length of time at sea-level, i e the base-line of complaints probably should be somewhat above zero On the other hand, there is no doubt that, with continuing stay in the field, we became so used to some abnormalities that we were scarcely aware of them, i e the recorded increase of complaints with altitude may have underestimated the increased abnormality of our experiences

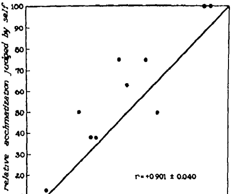

Fig 21 *a*

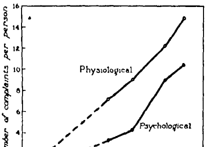

Fig 21 *b*

Fig 21*a* Relation between relative acclimatization of the individual as judged by himself and as judged by his fellows

Fig 21*b* Relative frequency of physiological and psychological complaints and abnormalities reported at different altitudes For nine normal men habituated to sea level

In any case, it is possible to arrange the Expedition members in order of subjective "fitness" from the relative frequency of complaints as indicated by the replies to the questionnaires When this is done it is evident that frequency of complaints is closely correlated with observed acclimatization (cf ratings in Table II)

It is obvious that self-judgement, both by direct and indirect methods, yields results not greatly different from the results of judgement by others As one might expect, the indirect method (replies to questionnaires) gives better results than the direct method of self-judgement and that, within the indirect method, self-observation of physiological abnormalities is a better guide than self-observation of psychological abnormalities

Elsewhere (McFarland 1937) we have reported the detailed objective analysis of sensory and mental function at high altitude as revealed by various tests It is of interest to note that impaired and disturbed psychological function at moderately high altitude is observed subjectively more readily than it can be detected by brief objective tests, at very high altitude (above 5000 m), however, this is no longer true and the subjective consciousness may not be aware of the effects of the altitude on psychological behaviour and ability This is in full agreement with the observations of engineers and others resident at altitudes up to 3500 m and of mountaineers and explorers who have climbed above 5000 m

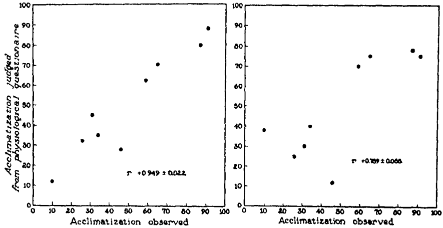

FIG 22

FIG 23

FIGS 22, 23 Relation between observed mean acclimatization to high altitude (2800–6140 m) and frequency of abnormalities and complaints recorded privately Fig 22 physiological complaints, fig 23 psychological complaints

GENERAL CONCLUSIONS AND SUMMARY

Individual variations in ability to acclimatize to high altitude were studied in the ten members of the International High Altitude Expedition over a period of about 4 months at altitudes up to 6140 m These men were all normal, healthy and vigorous

Relative acclimatization of the Expedition members was judged independently, on the basis of mutual observation, by the ten men for each of the high-altitude stations There was little disagreement as to the relative acclimatization of the different individuals

Comparison of the judgement of the individual by himself with that made by his fellows shows that there was a slight tendency to overestimate one's own acclimatization, but that self-judgement was rarely seriously in error

On the basis of questionnaire forms filled in privately by the Expedition members at each high-altitude station, it was found that the frequency of subjectively observed complaints and abnormalities, both physiological and psychological, was closely correlated with the relative imperfection of acclimatization

"Normal" individuals differ markedly in their ability to adjust to the rarefied air of high altitude and these differences have no obvious relation to general physical condition or past athletic ability The ability to adjust to high altitude appears to be related to a number of measurable characteristics at sea-level Of these characteristics, age, body size, basal pulse rate, alveolar gas tensions in the basal state, blood alkaline reserve, and oxygen capacity were important in our group Acclimatization to high altitude involves a number of factors so related to the foregoing characteristics that a consideration of these characteristics may be expected to give an acceptable basis for prediction as to ability to acclimatize to high altitude

For relatively normal men between the ages of 29 and 44, habituated to sea-level, it appears that youth, slow pulse, low–normal blood oxygen capacity, low alveolar oxygen pressure, high alveolar carbon dioxide pressure and high alkaline reserve at sea-level are favourable for acclimatization at high altitude

We found a good but not a perfect relation between observed relative acclimatization and our measured physiological characteristics at the same altitude Difficulty in obtaining this relation precisely is ascribed to the complex nature of the physiological adjustments which continue for many weeks at high altitude At high altitude the relative excellence of acclimatization is markedly correlated with a slow pulse (both basal and standing), with a small increase of pulse rate on rising from the prone position, and with an oxygen dissociation curve of the blood which is little changed from the sea-level position In partially acclimatized men there are slight but definite correlations between excellence of acclimatization and relatively low blood oxygen capacity, small diffusion gradient in the lung, and high saturation of the arterial blood with oxygen With more complete acclimatization these latter correlations tend to disappear

It is concluded that careful observation over a number of days by trained observers gives a better indication as to relative perfection of acclimatization than any laboratory measures yet devised

ACKNOWLEDGEMENTS

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SUPPLEMENT BY R A FISHER, F R S

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ON THE STATISTICAL TREATMENT OF THE RELATION BETWEEN SEA-LEVEL CHARACTERISTICS AND HIGH-ALTITUDE ACCLIMATIZATION

Seven of the sea-level characteristics of the members of the Expedition (figs 1-7) appear to show appreciable correlations with the subsequent acclimatization of these men at high altitude The combination of these seven variables in a formula so as to give the highest possible total correlation with acclimatization can be made by the method of least squares The formula has the form

$$\text{Acclim} = a + b(\text{pulse}) + c(pCO_2) + d(O_2\,\text{cap}) \text{ etc },$$

and the coefficients obtained by the method of least squares are given in the third column of Table A

TABLE A

Coefficient of pulse rate	Values used for fig 9	Least square values for 7 factors	4 factor formula	5 % fiducial limits
Pulse rate	−0 16	−0 7507	—	
pCO_2	−1 52	−2 534	—	
O_2 capacity	−3 10	−3 591	—	
Weight	−1 25	−0 9221	−1 628	(−0 720 to −2 532)
pO_2	−3 16	−3 590	−2 148	(−0 727 to −3 569)
Alkali reserve	+7 21	+6 472	+8 887	(5 171 to 12 603)
Age	−2 27	−2 207	−1 818	(−0 588 to −3 048)

The formula is calculated from, and tested by, only nine individuals With the coefficients of seven independent variables, and the absolute term, the formula contains eight adjustable constants Had one more sea level observation been included, however worthless this one might be, the observed estimates would have been given by the formula with perfect accuracy Since, actually, only seven variables have been used, there remains just one degree of freedom for departure from expectation Such departures usually furnish the basis for judging the precision of a formula, in this case the basis for such judgement is exceptionally narrow

The sum of the squares of the residues obtained by the application of these coefficients to the several observables is 13 174 For any coefficient to differ significantly from zero, the increase in the residual sum of squares due to dropping the corresponding variable out of the formula* would have to be about 160 (i e $12\ 706^2$) times as great as this last residual, or 2127 From the least square solution it appears that the increments due to omitting each of the seven variables severally are as shown in the second column below

TABLE B INCREMENT OF RESIDUAL SUM OF SQUARES DUE TO OMITTING EACH FACTOR SEPARATELY

	All factors	Omitting pulse rate	Omitting pulse rate and $p\mathrm{CO}_2$	Omitting pulse rate, $p\mathrm{CO}_2$ and oxygen capacity
Pulse rate	15 64	—	—	—
$p\mathrm{CO}_2$	27 05	11 40	—	—
O_2	70 72	64 05	65 51	—
Weight	58 36	311 85	339 16	655 77
$p\mathrm{O}_2$	190 03	218 24	523 54	464 93
Alkali reserve	210 72	352 23	355 15	1147 35
Age	531 71	516 29	509 40	444 65
Mean square residual	13 174	14 410	13 406	26 43
Needed for significance	2127	266 8	135 7	203 7

Hence it would appear that, as judged by the one degree of freedom of residual error, none of the seven coefficients differs significantly from zero at the 5 % level of significance

This does not mean that the formula is worthless, but that all the individual coefficients might be varied largely and, provided the other coefficients were suitably adjusted, the predictive value would not appreciably be impaired The formula in seven variables is, therefore, to a large extent arbitrary, and one or more of the variates used must certainly be redundant

This may be judged in another way The high ratio, 12 706, which for 5 % significance a coefficient must bear to its standard error, based on only one degree of freedom, is due to the fact that it is not improbable that this one component should happen to be very small in the sample of persons examined, compared with its value for other samples Consequently, to bring the probability to a convincing level, the contribution of the individual variables must be very large compared with the observed residue, if these are our sole sources of information on the magnitude of the discrepancies In this case, however, the manner in which ten independent judgements were combined to form the estimate of acclimatization provides a much more reliable estimate of the precision of this judgement, and also provides a lower limit below which its deviations from an acclimatization formula, derived from sea-level observations, cannot possibly fall

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* For technical details see *Statistical Methods*, especially Section 29 1 and Table IV, showing e g 12 706 as the 5% value of t for one degree of freedom

The total score assigned at each altitude to each subject is made up of 10 independent parts Its variance, therefore, is equal to 10 times the variance of these parts plus 90 times the average covariance of two different parts The method of scoring in which three subjects are given 5 marks less and three 5 marks more than the average, ensures that the sum of squares of deviations from the mean shall be $6 \times 25 = 150$ The observed sum of squares of deviations at any altitude, less 1500, may therefore be equated to 90 times the sum of products of the individual estimates But the average covariance of different independent estimates must be the true variance of the quantity estimated, and, subtracting the sum of squares of this quantity from the sum of squares of the aggregate estimate of it, we obtain the sum of squares of the aggregate errors

Thus if x_1, x_{10} are the aggregate scores given to 10 subjects at any altitude,

$$S(x) = 500,$$

$$S(x-50)^2 = A,$$

then $\dfrac{A-1500}{90}$ is the estimated mean sum of products of two individual assessments

Hence $$\frac{10}{9}(A-1500)$$

is an estimate of the true sum of squares of the total quantity assessed Subtracting from A we find

$$\frac{15{,}000-A}{9}$$

as the sum of squares of the errors of the total assessments

For the four altitudes used we have

Altitude km	A	$\dfrac{15{,}000-A}{9}$
2 81	7009	$887\ \dot{8}$
3 66	8700	700
4 71	8498	$722\ \dot{4}$
5 34, 6 14	9311	632 1
		2942 4

The total assessments at the four altitudes were averaged to give the acclimatization figure, so we may divide the total by 16 to obtain the sum of squares of deviations due to errors of estimation Since there are 9 subjects this should be further divided by 9, giving 20 434 as a minimal estimate of the variance by which a perfect formula ought to deviate from the observations

No contribution of less than four times this amount (80) could reasonably be accepted as significant, so that the contributions tested severally of pulse rate, pCO_2, weight and oxygen capacity indicate that at least one of these variables should be rejected

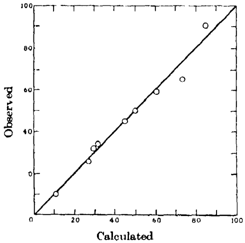

Fig 24 Prediction formula based on only four observables at sea level Compare fig 9, and the more accurate seven factor predictions of Table C

TABLE C EXPECTATIONS FROM LEAST SQUARES FORMULAE WITH SEVEN AND FOUR VARIABLES LEAST SQUARES FORMULA

	7 factors			4 factors	
	Obs	Exp	Diff	Exp	Diff
B	59	61 088	−2 088	59 527	−0 527
C	32	32 011	−0 011	29 675	+2 325
D	34	34 096	−0 096	31 863	+2 137
E	46	44 997	+1 003	45 632	+0 368
F	65	65 127	−0 127	72 688	−7 688
H	10	8 475	+1 525	10 761	−0 761
K	90	89 167	+0 833	84 221	+5 779
Mo	26	26 916	−0 916	27 496	−1 496
T	50	51 121	−1 121	50 136	−0 136

The effect of omitting pulse rate is shown in the third column of Table B The mean square residue is only slightly raised, from 13 171 to 14 408 Since this is based now on two degrees of freedom, the amount required for significance is much diminished, being only 266 8 in place of 2127 The three variables, weight, alkali reserve, and age now give significant regressions The contributions of oxygen capacity and of pCO_2 are, however, diminished to so small a value as to show that they also ought probably to be omitted

The fourth column shows the effect of leaving out both pulse rate and pCO_2 In addition to the three others pO_2 now certainly makes a significant contribution The mean square residue is, however, still lower than the value 20 434, which from the method of estimation appears to be a minimum value for the real errors of prediction Seeing that the omission of oxygen capacity only raises the mean square to 26 43 the advantage of retaining this variable is very doubtful The remaining four variables, used jointly, do, however, give a prediction with a standard error of about 5 on the scale used Fig 24 shows the observed acclimatization compared with the predicted values of Table C

For this formula I have added, in Table A, the coefficients and their 5 % fiducial limits, i e the limits within which the true value will lie in 95 cases out of 100

From Table C it appears that the largest discrepancies from the four factor formula are between the two climbers rated highest This suggests that with more assessors, or a more careful method of estimation in which each assessor places all the subjects in order of merit, F and K would be placed more nearly equal

575 1 591 53 595 724 *Carausius*

Transference of induced food-habit from parent to offspring III

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(*Communicated by E W MacBride, F R S —Received* 10 *March* 1938)

Introduction

The two previous communications in this series dealt with experiments designed to test the preference for, and ability to eat, ivy (as opposed to privet) in the stick insect *Carausius* (*Dixippus*) *morosus* It was shown that, under the conditions of the experiments, this insect increased its ability to eat ivy when reared on this food plant instead of on privet In the present paper these experiments are continued for two more generations and several other types of experiment are described which help to clear up some of the rather puzzling features of the earlier work

The methods employed are identical with those previously used, not because they cannot be improved upon, but because these later experiments had to be strictly comparable with the earlier ones

The experiments fall under two heads Presentation Tests and Preference Tests In presentation tests the newly hatched nymph is offered the experimental food plant for 24 hours If this is not eaten it is replaced by some of the parental food plant for 24 hours Then the experimental plant is again presented, this time for 48 hours A second refusal is again followed by a 24 hours presentation of parental food plant and a third presentation of the experimental food plant for 48 hours When at last the insect feeds on the experimental food plant the number of the presentation is recorded as the result of that experiment Since the insect only feeds at night, each 24 hours period represents one opportunity for a meal, thus, except for the first presentation, there is an alternation of two experimental and one parental food plant opportunities Newly hatched nymphs can only live for about 24 hours without food The alternations of parental food plant (which is always eaten) provides for the continued existence of the nymph (see tables for the very small number of deaths within experimental times) but does not allow any great degree of growth No nymph went through a period of ecdysis during the presentation tests even though 3 or 4 weeks old although the first instar is frequently over at the end of two weeks in normally fed insects

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[*] The tragic death of Miss Sladden occurred on 20 June 1937 when the final figures included in this paper were on the point of completion The preparation of the manuscript is therefore solely the responsibility of Mr Hewer It ought to be added that Mr Hewer deserves a considerable portion of the credit for Miss Sladden's previous papers as he devised the type of experiment which she carried out E W MacB

Preference tests consist in three presentations of both parental and experimental food plants at one and the same time so that the insect can choose between them If it accepts *either* parental *or* experimental food plant at all three presentations it is classed according to the food plant accepted If it accepts *both* parental *and* experimental food plants at *any one* presentation it is classed as "neutral" This latter ruling probably accounts for the rather large numbers of "neutral" insects, but it was considered that no "average" or "dominant preference could be stated from three presentations only and that a continuance of presentations would only result in the building up of a food preference, whether one existed previously or not It is for this reason that strict numerical comparison between experimental food-plant-preferring insects (in the preference tests) and those accepting the experimental food plant at the first presentation (in the presentation tests) cannot be made

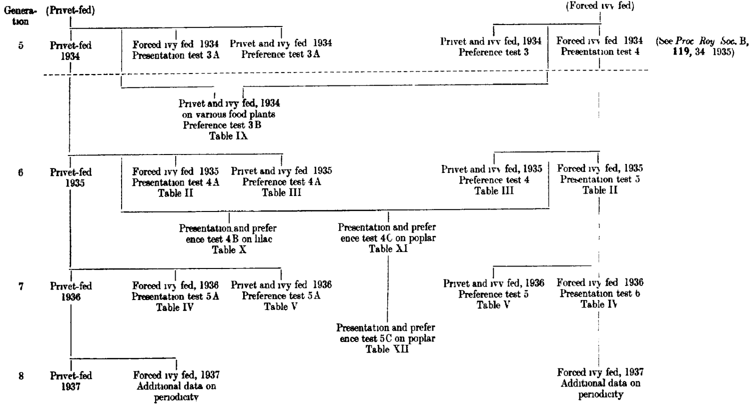
In order to make clear the relations of the various groups of insects employed in these experiments the genealogical table given in the second paper of the series (Sladden 1935, p 34) has been extended in Table I

PRESENTATION AND PREFERENCE TESTS

These tests are a direct continuation of those carried out on insects reared on privet and ivy respectively, the former being known as the controls and the latter as the forced ivy-fed

The results of the 6th generation tests are shown in Tables II and III One or two points call for comment Only one parental group of forced ivy-fed insects was used for the production of offspring submitted to the accurate recording of the presentation tests because a number of other experiments, to be described later, were undertaken at the same time and it would have been impossible to carry out either efficiently had the numbers been larger (Other parental groups of forced ivy-fed insects were used to produce offspring also forced ivy-fed but no detailed records were kept of these—see generation 7, Tables IV and V)

TABLE I


Generation
(Privet-fed)
(Forced ivy fed)
5
Privet-fed 1934
Forced ivy fed 1934 Presentation test 3A
Privet and ivy fed 1934 Preference test 3A
Privet and ivy fed, 1934 Preference test 3
Forced ivy fed 1934 Presentation test 4
(See *Proc Roy Soc.* B, **119**, 34 1935)
Privet and ivy fed, 1934 on various food plants Preference test 3B Table IX
6
Privet-fed 1935
Forced ivy fed 1935 Presentation test 4A Table II
Privet and ivy fed 1935 Preference test 4A Table III
Privet and ivy fed, 1935 Preference test 4 Table III
Forced ivy fed, 1935 Presentation test 5 Table II
Presentation and preference test 4B on lilac Table X
Presentation and preference test 4C on poplar Table XI
7
Privet-fed 1936
Forced ivy fed, 1936 Presentation test 5A Table IV
Privet and ivy fed 1936 Preference test 5A Table V
Privet and ivy fed, 1936 Preference test 5 Table V
Forced ivy fed 1936 Presentation test 6 Table IV
Presentation and preference test 5C on poplar Table XII
8
Privet-fed 1937
Forced ivy fed, 1937 Additional data on periodicity
Forced ivy fed, 1937 Additional data on periodicity

No emphasis can be laid on the 100 % acceptance at the first presentation because of the small number of insects used

Among the privet fed stock four groups were tested Fresh Forest Hill stock was introduced as a parallel strain to control stock of privet-fed insects for comparison All the insects used in the whole series of experiments were originally derived from this source (Sladden 1934, p 442) The three groups of control stock were used in order to test any possible variation due to emergence time That there is the possibility of this effect is shown by comparison of the times of the duration of the experiments shown in the first column of Table II This point will be dealt with fully later in the paper

TABLE II

PRESENTATION TEST 5 IVY-FED PARENTS, 1935

Duration of experiment	Parental presentation group	No tested	Presentation of ivy to progeny 1	2	3	4	5	Dead
Aug	1 1 1 1	100	100	—	—	—	—	0

PRESENTATION TEST 4A PRIVET FED PARENTS, 1935

Duration of experiment		No tested	Presentation of ivy to progeny 1	2	3	4	5	Dead
Sept –Oct	Forest Hill Stock	100	73	27	—	—	—	0
Aug –Sept	Control Group 1	100	92	8	—	—	—	0
Oct –Dec	Control Group 2	100	60	39	1	—	—	0
Feb –Mar	Control Group 3	100	10	65	19	5	1	0
	Percentage		58 8	34 8	5 0	1 2	0 2	

TABLE III

PREFERENCE TEST 4 IVY FED PARENTS, 1935

Parental presentation group	No tested	Privet	Neutral	Ivy	Dead
1 1 1 1	50	1	27	22	0

PREFERENCE TEST 4A PRIVET FED PARENTS 1935

	No tested	Privet	Neutral	Ivy	Dead
Forest Hill Stock	50	17	17	16	0
Control Group 1	50	5	20	25	0
Control Group 2	50	10	18	22	0

The preference tests give results according to expectations

In 1936 the seventh generation was similarly tested—the results are shown in Tables IV and V

TABLE IV

PRESENTATION TEST 6 IVY-FED PARENTS, 1936

Duration of experiment	Parental presentation group	No tested	Presentation of ivy to progeny 1	2	3	4	Dead
July–Sept	2 3 1 1	100	86	14	—	—	0
June–Aug	10 3 2 1	100	78	21	—	—	1
	Percentage		82 0	17 5	—	—	0 5

PRESENTATION TEST 5A PRIVET-FED PARENTS, 1936

Duration of experiment		No tested	Presentation of ivy to progeny 1	2	3	4	Dead
June–July	Forest Hill Stock	100	85	15	—	—	0
June–Aug	Control Group 1	100	86	13	1	—	0
June–Aug	Control Group 2	100	87	13	—	—	0
	Percentage		86 0	13 6	0 3	–	0

TABLE V

PREFERENCE TEST 5 IVY-FED PARENTS, 1936

Parental presentation group	No tested	Privet	Neutral	Ivy	Dead
2 3 1 1	50	2	15	33	0
10 3 2 1	50	4	21	24	1

PREFERENCE TEST 5A PRIVET-FED PARENTS, 1936

	No tested	Privet	Neutral	Ivy	Dead
Forest Hill Stock	50	4	27	19	0
Control Stock	50	6	27	17	0

The comparatively small numbers do not allow any far-reaching conclusions It will be seen however that both experimental and control insects accept ivy with almost equal readiness and that the period of the presentation tests extended over the months of June, July, August and September As shown in the section of this paper dealing with periodicity it is during these months that all the stick insects used show an equal ability to eat ivy That this is not peculiar to this test (presentation tests 5 and

4a) is seen from Table VII where insects (privet-fed) of three generations (1934, 1935 and 1936) show the same tendency

Reference to Table VI will show the consistency of this series of tests over six filial generations

TABLE VI

SUMMARY OF PRESENTATION TESTS

Duration of experiment	Filial generation	Privet fed parents Presentation of ivy									
		1	2	3	4	5	6	7	8	9	10
Jan –April	1	8 0	32 0	21 6	12 0	11 2	11 2	2 4	0 8	0 0	0 8
Nov –Jan	2	30 7	46 0	14 7	2 0	3 3	0 7	0 0	0 0	0 7	—
Nov –July	3	54 2	40 9	4 8	0 0	0 2	—	-	—	—	—
Sept –Nov	4	61 0	39 0	-	—	—	—	-	—	—	—
Mar –Aug	5	58 8	34 8	5 0	1 2	0 2	—	-	—	—	—
June–Aug	6	86 0	13 6	0 3	—	—	—	—	—	—	—

Duration of experiment	Filial generation	Ivy fed parents Presentation of ivy									
		1	2	3	4	5	6	7	8	9	10
	1										
Oct –April	2	78 25	18 99	1 5	0 12	0 12	—	—	—	—	—
Aug –May	3	93 7	6 2	0 1	—		—	—	—	—	—
July–Feb	4	90 3	9 5	0 3	—	—		—	—	-	—
Aug	5	100 0	—	—	—	—	—	—	—	-	—
July–Sept	6	82 0	17 5	—	—	—	—	—	—	—	—

SUMMARY OF PREFERENCE TESTS

Filial generation	Privet fed parents			Ivy fed parents		
	Privet	Neutral	Ivy	Privet	Neutral	Ivy
2	44 0	35 0	21 0	28 2	37 0	35 6
3	33 2	44 0	22 8	16 5	39 9	43 4
4	26 0	46 0	28 0	14 0	39 0	47 0
5	22 0	36 0	42 0	2 0	54 0	44 0
6	10 0	54 0	36 0	6 0	36 0	57 0

It will be seen however that the gradual concentration of privet-fed insects (controls) towards the first and second presentation groups which has been noted in a previous paper (Sladden 1935, pp 42, 43), reached its maximum in the fourth filial generation and the last two tests in the fifth and sixth filial generations show a return to the earlier conditions

This would be more marked in the fifth filial generation if a number had not been tested in August which the analysis of periodicity shows to be a month when food preferences are not well marked

TABLE VII

PRESENTATION GROUPS PRIVET-FED PARENTS

A Monthly acceptances each year

Month and year	1	2	3	4	5	6	7	8	9	10	Total
Jan 1933	8	25	8	2	1	1	2	—	—	—	47
Jan 1934	17	24	3	—	—	—	—	—	—	—	44
Feb 1932	3	20	20	13	9	10	1	—	—	—	76
Feb 1936	2	32	7	4	1	—	—	—	—	—	46
Mar 1932	6	20	7	2	5	3	—	—	—	—	43
Mar 1934	42	35	3	—	—	—	—	—	—	—	80
Mar 1936	8	33	12	1	—	—	—	—	—	—	54
April 1934	61	41	8	—	—	—	—	—	—	—	110
May 1934	66	39	2	1	—	—	—	—	—	—	108
June 1934	21	7	1	—	—	—	—	—	—	—	29
June 1936	61	14	—	—	—	—	—	—	—	—	75
July 1936	84	16	—	—	—	—	—	—	—	—	100
Aug 1935	61	5	—	—	—	—	—	—	—	—	66
Aug 1936	87	13	—	—	—	—	—	—	—	—	100
Sept 1934	24	6	—	—	—	—	—	—	—	—	30
Sept 1935	31	3	—	—	—	—	—	—	—	—	34
Oct 1934	20	18	—	—	—	—	—	—	—	—	38
Oct 1935	23	7	—	—	—	—	—	—	—	—	30
Nov 1932	2	9	2	—	2	1	—	—	1	—	17
Nov 1934	17	15	—	—	—	—	—	—	—	—	32
Nov 1935	29	21	—	—	—	—	—	—	—	—	50
Dec 1932	36	35	1	—	2	—	—	—	—	—	74
Dec 1933	13	21	2	—	—	—	—	—	—	—	36
Dec 1935	8	11	1	—	—	—	—	—	—	—	20

B Monthly totals

Month	1	2	3	4	5	6	7	8	9	10	Total
Jan	25	49	11	2	1	1	2	—	—	—	91
Feb	5	52	27	17	10	10	1	—	—	—	122
Mar	56	88	22	3	5	3	—	—	—	—	177
April	61	41	8	—	—	—	—	—	—	—	110
May	66	39	2	1	—	—	—	—	—	—	108
June	82	21	1	—	—	—	—	—	—	—	104
July	84	16	—	—	—	—	—	—	—	—	100
Aug	148	18	—	—	—	—	—	—	—	—	166
Sept	55	9	—	—	—	—	—	—	—	—	64
Oct	43	25	—	—	—	—	—	—	—	—	68
Nov	48	45	2	—	2	1	—	—	1	—	99
Dec	57	67	4	—	2	—	—	—	—	—	130
											1339

TABLE VII (*continued*)

C Mean presentation times and standard deviations

	M P T	S D
Jan	2 08	± 0 78
Feb	3 07	± 1 40
Mar	1 99	± 1 03
April	1 52	± 0 63
May	1 43	± 0 58
June	1 22	± 0 44
July	1 16	± 0 37
Aug	1 11	± 0 32
Sept	1 14	± 0 35
Oct	1 37	± 0 48
Nov	1 71	± 1 36
Dec	1 64	± 0 96

TABLE VIII PRESENTATION GROUPS IVY-FED PARENTS

A Monthly acceptances each year

Month and year	1	2	3	4	5	Total
Jan 1933	69	11	—	—	—	80
Jan 1934	24	5	—	—	—	29
Jan 1935	21	5		—	—	26
Feb 1933	68	9	—	—	—	77
Feb 1934	15	2	—	—	—	17
Mar 1933	19	14	—	—	—	33
Mar 1934	13	1	—	—	—	14
Mar 1937	9	9	—	—	—	18
April 1933	7	7	—	—	—	14
April 1934	18	7	—	—	—	25
May 1934	9	4	—	—	—	13
June 1936	9	3	—	—	—	12
June 1937	56	6		—	—	62
July 1934	12	—	—	—	—	12
July 1936	74	25	—	—	—	99
Aug 1933	65	3	—	—	—	68
Aug 1934	94	6	—	—	—	100
Aug 1935	100	—	—	—		100
Aug 1936	65	5	—	—	—	70
Sept 1933	300	20	—	—	—	320
Sept 1934	91	2	1	—	—	94
Sept 1936	16	1	—	—	—	17
Oct 1933	298	14	—	—	—	312
Oct 1934	53	7	—	—	—	60
Nov 1932	44	16	2	1	—	63
Nov 1933	145	6	—	—	—	151
Nov 1934	36	11	—	—	—	47
Dec 1932	74	10	1	—	—	85
Dec 1933	73	8	—	—	—	81
Dec 1934	51	5	—	—	—	56

TABLE VIII (*continued*)

B Monthly totals

Month	1	2	3	4	5	Total
Jan	114	21	—	—	—	135
Feb	83	11	—	—	—	94
Mar	41	24	—	—	—	65
April	25	14	—	—	—	39
May	9	4	—	—	—	13
June	65	9	—	—	—	74
July	86	25	—	—	—	111
Aug	324	14	—	—	—	338
Sept	407	23	1	—	—	431
Oct	351	21	—	—	—	372
Nov	225	33	2	1	—	261
Dec	198	23	1	—	—	222
						2155

C Mean presentation times and standard deviations

	M P T	S D
Jan	1 16	± 0 36
Feb	1 12	± 0 32
Mar	1 37	± 0 49
April	1 36	± 0 48
May	1 31	± 0 48
June	1 12	± 0 33
July	1 23	± 0 41
Aug	1 04	± 0 20
Sept	1 06	± 0 23
Oct	1 06	± 0 23
Nov	1 15	± 0 41
Dec	1 11	± 0 33

TABLE IX PREFERENCE TEST 3B 1934

Experimental food rose

	No tested	Ivy	Neutral	Rose	Dead
Progeny of ivy fed parents	250	71	88	90	1
[Per 50		14 2	17 6	18 0	0 2]
		Privet	Neutral	Rose	
Progeny of privet fed parents	50	4	26	20	0

Experimental food lilac

	No tested	Ivy	Neutral	Lilac	Dead
Progeny of ivy fed parents	250	46	77	127	0
[Per 50		9 2	15 4	25 4	0]
		Privet	Neutral	Lilac	
Progeny of privet fed parents	50	3	19	28	0

TABLE IX (*continued*)

Experimental food fuchsia

	No tested	Ivy	Neutral	Fuchsia	Dead
Progeny of ivy fed parents	250	89	81	80	0
[Per 50		17 8	16 2	16 0	0]
		Privet	Neutral	Fuchsia	
Progeny of privet fed parents	50	14	24	12	0

Experimental food blackberry

	No tested	Ivy	Neutral	Blackberry	Dead
Progeny of ivy fed parents	250	29	60	161	0
[Per 50		5 8	12 0	32 2	0]
		Privet	Neutral	Blackberry	
Progeny of privet fed parents	50	5	13	32	0

Experimental food poplar

	No tested	Ivy	Neutral	Poplar	Dead
Progeny of ivy fed parents	250	249	0	0	1
[Per 50		49 8	0	0	0 2]
		Privet	Neutral	Poplar	
Progeny of privet fed parents	50	50	0	0	0

TABLE X

PRESENTATION TEST 4B IVY FED PARENTS, 1935

Parental presentation group	No tested	Presentation of lilac to progeny				Dead
		1	2	3	4	
2 3 1 1	100	92	8	—	—	0
10 3 2 1	100	96	4	—	—	0

PRIVET FED PARENTS, 1935

Forest Hill Stock	100	96	4	—		0
Control Stock	100	95	5	–	—	0

PREFERENCE TEST 4B IVY-FED PARENTS, 1935

Parental presentation group	No tested	Ivy	Neutral	Lilac	Dead
2 3 1 1	50	10	8	32	0
10 3 2 1	50	8	16	26	0
Total	100	18	24	58	0

PRIVET FED PARENTS, 1935

Forest Hill Stock	50	2	15	33	0
Control Stock	50	8	17	25	0
Total	100	10	32	58	0

TABLE XI

PRESENTATION TEST 4C IVY-FED PARENTS, 1935

Parental presentation group	No tested	Presentations of poplar to progeny 1	2	3	4	5	6	7	8	9	10	Refused	Dead
2 3 1 1	100	0	9	4	7	2	2	3	3	0	2	53	15
10 3 2 1	100	1	3	0	5	3	2	2	3	3	3	53	22
Total	200	1	12	4	12	5	4	5	6	3	5	106	37
						57						143	

PRIVET-FED PARENTS, 1935

		1	2	3	4	5	6	7	8	9	10	Refused	Dead
Forest Hill Stock	100	0	2	6	5	3	1	5	1	2	1	68	6
Control Stock	100	2	2	3	3	1	3	1	0	1	1	67	16
Total	200	2	4	9	8	4	4	6	1	3	2	135	22
						43						157	

PREFERENCE TEST 4C IVY-FED PARENTS, 1935

Parental presentation group	No tested	Ivy	Neutral	Poplar
2 3 1 1	50	50	0	0
10 3 2 1	50	50	0	0

PRIVET FED PARENTS, 1935

		Privet	Neutral	Poplar
Forest Hill Stock	50	50	0	0
Control Stock	50	50	0	0

PERIODICITY

Previously it had been suggested that the concentration was due to seasonal changes (Sladden 1935, pp 43, 44) This has been the subject of further investigation Fortunately, all records, with the exception of a few in 1932, contained data about the dates of emergence and consequent testing It was therefore possible to reorientate all the presentation tests figures in the monthly periods irrespective of parentage or year These data are set out in Tables VII (ivy fed parents) and VIII (privet-fed parents)

Each table contains the yearly composition of the monthly groupings, the monthly totals in presentation groups and lastly the mean presentation times expressed as decimals of group numbers

During 1936 and 1937 further presentation tests were carried out in order to increase the totals for certain months The totals are now reasonably high, with the exception of that for the month of May among the ivy-fed insect data No emergences among these insects have occurred for several years in the month of May

Referring first to the privet-fed stock (controls) the periodicity postulated is shown very obviously not only in the percentages accepting ivy at

TABLE XII

PRESENTATION TEST 5C POPLAR FED PARENTS, 1936

Parental presentation group	No tested	Presentation groups 1–10	Refused 10th presentation	Dead
(2 3 1 1) 2	20	0	4	16
(2 3 1 1) 4	50	0	39	11
(10 3 2 1) 4	30	0	26	4
(10 3 2 1) 7	50	0	40	10
(Forest Hill) 3	95	0	74	21
(Forest Hill) 7	100	0	87	13
Total	345	0	270	75

PREFERENCE TEST 5C POPLAR-FED PARENTS, 1936

Parental presentation group	No tested	Ivy	Neutral	Poplar	Dead
(2 3 1 1) 2	13	9	0	0	4
(2 3 1 1) 4	50	50	0	0	0
(10 3 2 1) 4	16	16	0	0	0
(10 3 2 1) 7	50	48	0	0	2
	No tested	Privet	Neutral	Poplar	Dead
(Forest Hill) 3	50	50	0	0	0
(Forest Hill) 7	50	50	0	0	0

Indices outside brackets refer to poplar presentation groups

TABLE XIII

Stock	Eggs selected	Hatched	Used	% selected/ hatched	% selected/ used
(2 3 1 1) 2	300	40	33	13 3	11 0
(2 3 1 1) 4	315	164	100	52 1	31 7
(10 3 2 1) 4	225	46	46	20 4	20 4
(10 3 2 1) 7	225	157	100	69 8	44 4
(Forest Hill) 3	330	171	145	51 8	43 9
(Forest Hill) 7	255	166	150	65 1	58 8
Totals	1650	744	574	Means 45 1	34 8
	Cf Table X, Sladden (1935) which gives				
Totals	4050	3660	2600	Means 90 4	71 0

the first presentation (as opposed to those accepting ivy at the second or third) but also in the number of presentations required to induce some insects to accept ivy during the winter and spring The correspondence between the results for February and March in 1932 and 1936 is notable as indicating the probable validity of the figures, consistent as they are with the January figures of 1932 and 1933

Examination of these tables can leave no doubt that a periodicity exists either in the palatability of the food plant or in the habits of the insect There is no direct evidence to distinguish between these two possibilities, but this does not matter since we are concerned solely with the elucidation of the anomalous results obtained with the controls from year to year

It will be seen that there is an approximation of observed data on both experimental and control insects during the months of June, July and August In fact it is quite conceivable that a repetition of these experiments carried out over these three months would produce results, mathematically significant, in direct contradiction to those obtained by us during other periods of the year It is to be regarded as fortunate that our original experiments were carried out during the time of year when the differences in behaviour between the privet-fed and ivy-fed forms is at its maximum *This point must be borne in mind should these experiments be repeated*

TESTS ON OTHER FOOD PLANTS

In the course of the experiments previously communicated it seemed possible to us that the increased ability to eat ivy shown by the ivy-fed stock might not be specific but merely an expression of a more generalized ability to eat any other food plant

With a view to testing this view a number of preference tests were made with five different food plants, some of which had already been reported as possible foods for the insect The results of these tests are set out in Table IX It will be noted that the ivy fed stock were tested with ivy and the experimental food plant, and the privet-fed stock tested with privet and the same food plant This appeared to be the only way to make comparable tests The large number (250) progeny of ivy fed parents tested is due to the use of five different parental groups each providing fifty experimental progeny There was no significant difference between any of these groups so that the average per fifty is quite justified

The following points emerge

(1) Poplar is consistently refused and is the only food plant of the five to be more distasteful to the insect than ivy or privet

(2) Rose, lilac and blackberry appear to be more readily eaten than ivy or privet

(3) Fuchsia appears to be at about the same degree of "preference" as ivy and privet

(4) Leaving "poplar" on one side, in all the other experiments the progeny of ivy-fed parents appear to "prefer ivy to the experimental food plant *more* than the progeny of privet-fed parents "prefer" privet

There is thus no evidence that the induction of an ivy-feeding habit has "broken" a privet feeding habit since privet is certainly not one of the most palatable food plants to the insect

In fact to speak in such terms is obviously inconsistent with the delicacy of the process which has really taken place The change in food habit is, in fact, extremely small and only detectable by a method of presentation and preference tests such as we have employed

As an example of this delicacy we may quote a further experiment carried out using lilac as the experimental food plant In Table X are set out the results of presentation and preference tests on the progeny of both ivy-fed and privet-fed parents

The results of presentation test 4 B are such that any further generations for purposes of testing are little good since the percentage taking lilac at the first presentation is very high while those preferring lilac to ivy is over 50 % (preference test 4 B)

Preference test 4 B is practically a repetition of the preference test in lilac in Table IX and the figures will be seen to agree very well

Another example, in the opposite direction, is shown in the presentation and preference tests in poplar (Tables XI and XII) In these tables two generations are shown and the most noticeable feature of the experiment as a whole is the complete failure to induce the insects to increase their ability to eat poplar

After presentation test 4 C it hardly seemed worth while to continue the experiment since so many individuals had either died or refused poplar for ten presentations This limit had to be imposed as otherwise it became impossible to keep adequate records of the individuals tested

The experiment was however continued for a second generation to see whether selection was taking place In Table XII it will be seen that none of the 345 progeny tested accepted poplar during the first ten presentations The death rate was again high It is possible to account for this on the grounds that a modification of feeding habit towards "ability to eat poplar" is outside the range of adaptability of the insect Its repugnance for this food is very clearly shown in all the preference tests

Lastly it may be noted that these experiments on poplar were accompanied by a great decrease in the fecundity of the parents and in the viability of the eggs This is shown in Table XIII with a comparison with totals from normal batches of eggs

CONCLUSIONS

This series of experiments is now brought to an end, and it is very doubtful whether they could ever be repeated unless someone could be found willing to devote himself throughout the year to the tedious recording and experimentation carried out by Miss Dorothy Sladden, over a period of some six years

The conclusions appear to be fairly clear Two sets of stick insects, one reared on privet in every generation but tested for their ability to accept ivy by presentation and preference tests on samples of each generation, the other reared on ivy in every generation after having been tested for their ability to accept it by identical tests, have demonstrated different relative tendencies to accept ivy as a food plant

Those forced to eat ivy from the first generation onwards rapidly developed an increased ability to accept this food plant, an ability which is comparatively little affected by the time of year when the test is made

Those reared on privet continuously displayed during the first four filial generations an increased ability to accept ivy but to a much less extent than did the ivy-fed stock Further this increased ability to accept ivy appears to be a function of the time of year when the tests are made, since not only do the fifth and sixth filial generations show a *decrease* in the ability to accept ivy but analysis of the results on a monthly basis displays a distinct annual periodicity only slightly and hardly significantly shown by the ivy fed stock

It is therefore difficult to escape the conclusion that the forced ivy-feeding has induced the increased ability to accept ivy in succeeding generations in this parthenogenetic insect It is a matter of the deepest regret that the work cannot be carried on and repeated with insects reproducing by sperm and egg

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The effect of anterior pituitary extracts on established lactation in the cow

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In 1928 Stricker and Grueter found that the administration of a crude anterior pituitary extract to rabbits, which had been ovariectomized during pseudopregnancy, resulted in the secretion of milk This result was of fundamental importance as it suggested that the stimulus to the onset of lactation after parturition was the secretion of a substance by the pituitary gland, and not merely the removal of the stimuli which cause growth of the mammary gland during pregnancy The results of Stricker and Grueter have been widely confirmed and at the present time the theory is generally accepted that initiation and maintenance of lactation are controlled by the anterior pituitary gland The chief evidence for this theory, which has been adequately reviewed recently by Nelson (1936), is, briefly, that normal lactation does not occur spontaneously in the absence of the pituitary gland, although the secretion of milk may, in suitable circumstances, follow the administration of anterior pituitary extracts to normal or hypophysectomized animals

The simplest interpretation of the evidence suggests that the anterior pituitary gland secretes a "lactation" or ' lactogenic" hormone, and until recently this view had been generally held Evidence in its support has been provided by Riddle and others (see Nelson for references) who have prepared relatively purified anterior lobe extracts capable of inducing copious secretion of milk in experimental animals under suitable conditions Riddle has named the active principle "prolactin" and has shown that preparations of prolactin possess the ability to cause growth of the pigeon crop-gland In view of the accumulation of evidence suggesting that stimulation of growth of the pigeon crop gland is a specific property of prolactin, methods of assay of prolactin have almost entirely been based on the pigeon crop-gland test It is therefore convenient, as will be done in the present communication, to use the name prolactin to describe the substance present in anterior pituitary extracts which stimulates growth of the pigeon crop-gland, without prejudice to the question of whether or not all preparations of prolactin are lactogenic

During the past year evidence has accumulated which indicates that comparatively purified preparations of prolactin are incapable of promoting milk secretion in hypophysectomized animals, although, in most instances, crude anterior lobe extracts are capable of so doing From the work of Nelson and Gaunt (1937) and of Gomez and Turner (1937*a*, *b*) it appears that, in addition to prolactin, the administration of either adrenal cortex hormone or anterior pituitary adrenotropic hormone is necessary for the initiation and maintenance of lactation in hypophysectomized animals It has further been suggested that the carbohydrate metabolism hormone of the anterior pituitary gland is also necessary (Gomez and Turner 1937*b*) The presence of the "anti-insulin" or "glycotropic" (Young 1936*a*) factor of the anterior lobe in preparations of prolactin, as normally obtained, is probably of significance in this connexion (Young 1936*b*) The evidence at present available therefore suggests that prolactin is by no means the only anterior pituitary substance which is concerned in the control of lactation

The important question of the composition of the fluid secreted by the mammary gland under the stimulus of pituitary preparations has not received sufficient attention Bergman and Turner (1937) compared the composition of the milk secreted by pseudopregnant rabbits under the stimulus of anterior lobe extracts with that of normal rabbit colostrum and concluded that the two were similar in fat and lactose contents, but few studies of this type have been reported Many authors have regarded the formation of a few drops of milk-like fluid as indicative of "lactogenic" activity of the preparation injected, a conclusion which is not altogether satisfactory As attempts have been made to stimulate deficient milk secretion in women by anterior lobe therapy, it seems desirable that much more attention should be paid to the composition of the milk secreted For such an investigation the cow is very suitable since much is known about the composition of cows' milk under different conditions, and large amounts can readily be obtained

Azimov and his collaborators (Azimov, Kruze, Skaržinskaja, Mahova, and Fominskaja, 1936, Azimov and Krouze 1937) found that a single injection of a small amount of a crude alkaline extract of anterior pituitary lobe caused a substantial temporary increase in the milk yield of cows in declining lactation The composition of the milk secreted during the period of stimulation was normal

In the present investigation cows in declining lactation have been used In most instances the influence of a single injection of different anterior pituitary preparations on milk yield and composition was studied, but in two experiments the effect of daily injections of prolactin on milk yield etc was determined Some results from one of these experiments have already been briefly recorded (Folley and Young 1937) It was hoped, by investigations with suitably standardized extracts, to throw some light on the question of the relationship of the anterior lobe factors concerned with carbohydrate metabolism to those controlling lactation For this purpose preparations of prolactin having high or low glycotropic (anti-insulin) activities were used as well as preparations which were known to possess diabetogenic activity in the normal dog Glycotropic activity is determined on the basis of ability to abolish the hypoglycaemic action of intravenous insulin in the intact fasting rabbit without significantly affecting the resting blood-sugar level and it must be stressed that an anterior lobe preparation may possess substantial glycotropic activity without possessing the ability to induce the symptoms of diabetes on administration to a normal dog (Young 1936*a* 1938*b*)

The administration of thyroxine is known to increase the milk yield of lactating cows (Graham 1934*a*, *b* Folley and White 1936) and it was also of interest to determine whether or not the administration of anterior pituitary thyrotropic preparations would stimulate milk production in the cow

METHODS

Experimental animals

Altogether thirty-three cows of the Dairy Shorthorn breed were used in the present investigation, nineteen of these belonged to the herd of the Reading University farm, and fourteen to the herd of the National Institute for Research in Dairying The feeding of the animals was that usual in this country for dairy cows under good management The cows were milked twice daily at approximately 7 a m and 4 p m , the milk from each milking being weighed on a spring balance to 0 25 lb All injections were given subcutaneously

In most of the experiments some of the animals used were pregnant, but care was taken to ensure that each member of the control group of cows resembled its counterpart in the experimental group as closely as possible with regard to stage of lactation, number of lactations, time since mating if pregnant, etc

Anterior pituitary gland extracts

(*a*) *Preparation* All these were prepared from ox anterior lobe tissue Crude saline extract was prepared at low temperature from fresh ox anterior lobe by the method described by Young (1938*a*) The extract was stored at 0° C and used within 2 days of preparation The volume of the extract was so adjusted that 3 c c of extract was equivalent to 1 g of fresh gland tissue

"Prolactin-C" and "Thyrotropic-C", i e preparations obtained at low temperature from fresh ox glands, were prepared by the method described by Young (1938*b*) The "Thyrotropic-C" of the present paper is the same as the "non-Prolactin-C" of the earlier communication These preparations were stored in a frozen condition at − 11° C for some weeks before use Their volume was so adjusted that 3 c c of each extract were equivalent to 1 g of fresh gland tissue

Two samples of partially purified prolactin (preparations 32 C and P_3) were prepared from commercial (B D H Ltd) acetone-desiccated ox anterior lobe by the modification of the method of Bates and Riddle (1935) described by Young (1938*b*) As these two preparations were of the same type, they will be referred to as Prolactin 1 and Prolactin 2 respectively in the present communication

A thyrotropic preparation (50 D_1) was also obtained from commercial dried gland by the method of Young (1938*b*) This preparation will be designated as Thyrotropic 1 in the present paper

(*b*) *Pigeon crop-gland assays* All the preparations used were assayed for pigeon crop-gland stimulating activity by the method described by Rowlands (1937) The results are given in Table I

It will be seen from Table I that no pigeon crop gland stimulating activity could be found in Thyrotropic 1 or Thyrotropic-C by the method used

TABLE I PROLACTIN ASSAYS ACCORDING TO THE METHOD OF ROWLANDS (1937)

Preparation	Amount injected daily	Mean crop gland increase g /300 g bird
Crude saline extract	0 1 c c	2 7
Prolactin 1	1 0 mg	4 6
Prolactin 2	3 0 mg	5 0
Prolactin C	0 2 c c	3 1
Thyrotropic 1	20 mg	0 0
Thyrotropic C	2 0 c c	0 0

(*c*) *Thyrotropic activity assays* The preparations were assayed for thyrotropic activity by the method of Rowlands and Parkes (1934), with the results recorded in Table II According to Heyl and Laqueur (1935) the substance which induces enlargement of the thyroid gland and that which induces increased secretion of the thyroid hormone are not identical Our preparations have been assayed only by the method utilizing the enlargement of the thyroid gland of the immature guinea-pig In the present communication, therefore, the thyrotropic activity is that inducing increase in size of the thyroid gland and not necessarily causing increased secretion of the gland Table II shows that no thyrotropic activity could be found in Prolactin I by the method of assay used, and Prolactin 2 and Prolactin-C each contained only a trace of thyrotropically active contaminant

TABLE II THYROTROPIC ASSAYS ACCORDING TO THE METHOD OF ROWLANDS AND PARKES (1934)

Preparation	Amount injected daily	Increase in weight of thyroid gland mg
Crude saline extract	0 5 c c	+40
Prolactin 1	20 mg	− 1
Prolactin 2	10 mg	+ 9
Prolactin C	0 5 c c	+ 5
Thyrotropic 1	1 mg	+17
Thyrotropic C	0 5 c c	+43

(*d*) *Determination of glycotropic activity* The glycotropic ("anti insulin") activity of the pituitary preparations was assessed on the basis of the influence, in intact rabbits, of two injections of extract on the hypoglycaemic action of 2 units of intravenously administered insulin, injected 4½ hr after the second injection of pituitary extract The details of the procedure are given by Young (1938*b*) Under the conditions of this test the resting blood-sugar level of the rabbits is not significantly influenced by the injection of the active pituitary extracts, although the hypoglycaemic action of the insulin may be completely abolished

Table III gives the amount of the different preparations required, in each of two injections, to abolish, or nearly to abolish, the hypoglycaemic action of 2 units of insulin in intact rabbits under the conditions of the above test

(*e*) *Diabetogenic activity* Only two of the preparations used (crude saline extract and Thyrotropic C) possessed true diabetogenic activity, i e the daily administration to normal dogs of suitable doses of either of these preparations only, induced the appearance of the symptoms of diabetes mellitus (see Young 1938*b*)

(*f*) *Relative biological activities of the preparations used* In Table IV is given a summary of the relative biological activities of the different anterior pituitary preparations used Comparisons of the activities of Prolactin-C and Thyrotropic-C with the other preparations are made on the basis of the relative weights of anterior lobe tissue required for the preparation of amounts of extract having comparable biological activities

TABLE III THE AMOUNT OF ANTERIOR PITUITARY EXTRACT REQUIRED IN EACH OF TWO INJECTIONS TO ABOLISH, OR NEARLY TO ABOLISH, THE HYPOGLYCAEMIC ACTION OF INSULIN UNDER THE CONDITIONS DESCRIBED

Preparation	Amount required
Crude saline extract	4 c c
Prolactin 1	100 mg
Prolactin 2	150 mg
Prolactin C	6 c c
Thyrotropic 1	—*
Thyrotropic C	9 c c

* A satisfactory glycotropic assay could not be carried out on this preparation owing to posterior lobe contamination (see below) Assays on similar preparations containing no detectable oxytocin suggest that two doses each of 150–200 mg would have been required nearly to abolish the hypoglycaemic action of insulin in the test

TABLE IV RELATIVE BIOLOGICAL ACTIVITIES OF THE PREPARATIONS USED

Preparation	Prolactin	Glycotropic activity	Thyrotropic activity	Diabetogenic activity
Crude saline extract	+ + +	+ + +	+ + +	+ + +
Prolactin 1	+ +	+	0	0
Prolactin 2	+ +	+	Trace	0
Prolactin C	+ +	+ + +	Trace	0
Thyrotropic 1	0	(+)?	+ +	0
Thyrotropic C	0	+ +	+ + +	+ +

Although no thyrotropic activity could be detected in Prolactin 1 preparation, and no pigeon crop-gland stimulating activity was found in Thyrotropic 1 and Thyrotropic-C, it cannot be assumed that these preparations were entirely free from substances having these activities All that can be said is that no detectable activity was found by the methods in use in our laboratories

Table IV indicates that Prolactin preparations 1 and 2 have pigeon crop-gland stimulating activities similar to that of Prolactin-C, but that the glycotropic activity of the Prolactin-C preparation (obtained from fresh gland) is much greater than that of Prolactin 1 or of Prolactin 2, both of which preparations were obtained from commercial dried anterior lobe

Posterior lobe contamination of anterior lobe extracts

Though there is little evidence that extracts of posterior lobe influence the secretory activity of the mammary gland parenchyma, in view of the fact that injections of posterior pituitary extracts have been shown to stimulate the release of preformed milk from the mammary gland (Hammond 1913, Turner and Slaughter 1930), it was considered advisable to assay the anterior lobe extracts used in the present investigation for oxytocic activity, using the virgin guinea-pig uterus method The results of the assays, which are given in Table V, show that, with the exception of Thyrotropic 1, the extracts contained only negligible amounts of substances causing contraction of uterine muscle The extracts were heated on a boiling water bath for 2 minutes in 0 25% acetic acid before the assays of oxytocic activity were made, in order to exclude some, though probably not all, non-specific substances having an action on the guinea-pig uterus similar to that of oxytocin

TABLE V POSTERIOR LOBE ASSAYS ON ANTERIOR LOBE EXTRACTS

Preparation	Oxytocic activity international units
Crude saline extract	1 c c = 0 01 unit
Prolactin 1	1 mg = 0 0002 unit
Prolactin C	1 c c = 0 005 unit
Thyrotropic 1	1 mg = 0 25 unit
Thyrotropic C	1 c c = 0 006 unit

Sampling of milk In most experiments samples were taken from the morning and evening milkings for the purpose of chemical analysis The samples of individual cows were analysed separately so that the reaction of each animal to the experimental procedure could be determined From the yields and analyses of the milk from individual cows in a group, a "weighted" mean content of the solids in the milk of the group could be calculated

In all experiments control analyses on the milk were made over a period of at least a week before the pituitary injections were given

Milk analyses Milk fat content was determined by the Gerber method, and total dry matter content by evaporation at approximately 105° C From the data thus obtained the percentage of non-fatty solids in the "fat free milk serum" could be calculated (see Bartlett 1933–4), the correction for milk fat content being made in cases where alterations in the amount of this constituent rendered this correction desirable Milk phosphatase (Phosphomonoesterase A_1 in the classification of Folley and Kay, 1936*a*) was determined by the method of King and Armstrong (1934), lactose by the method of Blackwood (1934), chlorides by the method of Davies (1932) and nitrogen partition by the method described by Moir (1931)

Blood-sugar estimations In one of the experiments described below (Exp 7) blood samples were taken at frequent intervals from the jugular veins of the cows, for blood-sugar determinations Since, from the nature of the experiments, it was not desirable to have the animals in a fasting condition when blood samples were taken, the samples were withdrawn at the same hour (9 45 a m) each day, at approximately the same time after the morning milking and accompanying meal The blood was drawn into cooled, oxalated tubes which were immersed in ice water until deproteinization of the blood sample was effected The proteins were always precipitated within 20 minutes of sampling, and the reducing substances in the tungstic acid filtrate determined before and after fermentation, by means of Somogyi's reagent 2 (see Peters and Van Slyke 1931) In this way the "true" blood-sugar content was determined

RESULTS

Stimulation of milk production by a single injection of a crude saline extract of anterior pituitary gland

Two groups of six cows (Groups A and B) were used in these experiments, two consecutive experiments (Nos 1 and 2) being carried out After a preliminary control period of 10 days all the cows in Group A were given one subcutaneous injection of 15 c c of a crude saline extract (equivalent to 5 g of fresh anterior lobe), while at the same time the cows in Group B received similar injections of an extract of fresh beef liver prepared in the same way as the anterior pituitary extract After an interval of 21 days the cows of Group B each received one subcutaneous injection of 30 c c of crude saline anterior pituitary extract (equivalent to 10 g of fresh gland), while the animals in Group A received equivalent control injections of liver extract The effects resulting from the first injection had subsided after about 6 days, so that 10 at least of the remaining 15 days before the second injections may safely be taken as a preliminary control period for that experiment

Yield of milk and milk solids The aggregate daily milk yields of both groups of cows together with the total amounts of milk fat and non-fatty solids secreted by each group on approximately alternate days are plotted in figs 1 (Exp 1) and 2 (Exp 2)

It will be seen that in both experiments the single injection of extract induced an immediate increase in milk yield In the first experiment the response reached its peak on the day following the injections, while in the second experiment, when double the previous dose was administered, the peak response came on the second day following the injections In both cases the effect subsided rapidly During the control periods the aggregate milk yield curves for both groups of cows show the regular decline characteristic of cows in the later stages of lactation Over short periods declining lactation curves usually approximate to straight lines, so that for each experiment an approximate estimate of the milk yields likely to have been given by the experimental cows, had they not received the injections (hereinafter called the "basal" yields), can be obtained graphically from the straight line, which fits portions of their lactation curve immediately preceding and following the response period Both in figs 1 and 2 the best straight line was fitted by the method of least squares to all points on the milk yield curve for the pituitary injected cows, save those which were judged to fall within the response period Doubtful cases at the end of the response period were included in the latter category This procedure enables an approximate quantitative estimate of the extra milk produced in response to the injection to be made, and also an estimate of the duration of the response to the injections, i e the time which elapses before the lactation curve returns to its normal course

Values for the duration of response, aggregate "basal" milk yield, and mean percentage increase for the whole response period in both experiments are shown in Table VI

TABLE VI

Cows (group of six)	Injections	Duration of response days	Aggregate "basal" milk yield lb	Actual milk yield lb	Increase due to injections lb	Total increase as % of "basal" yield
Group A (Exp 1)	15 c c of crude saline extract	5	751	828¼	77¼	10 3
Group B (Exp 2)	30 c c of crude saline extract	6	787	878	91	11 6

The results in Table VI show that an injection of 30 c c of extract did not stimulate milk yield to a significantly greater degree than an injection of half that amount, which suggests that the response evoked by the lower dose is the maximum possible under the conditions of these experiments

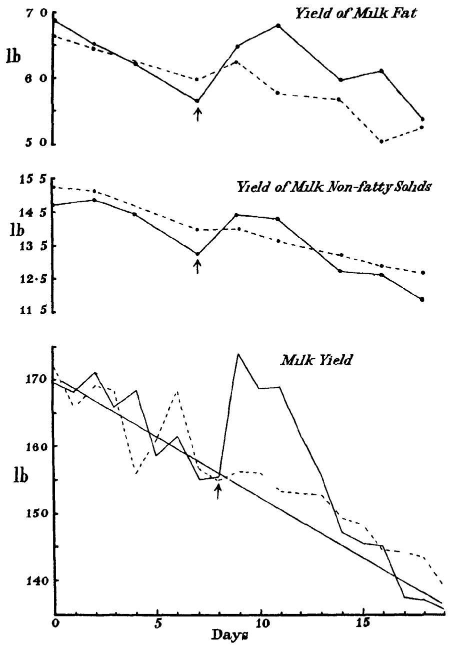

FIG 1 Aggregate yields of milk, milk non fatty solids and milk fat

—— Group A (cows receiving injections of saline extract of anterior pituitary gland)

- - - Group B (cows receiving injections of saline extract of liver)

↑ signifies last observations before injection

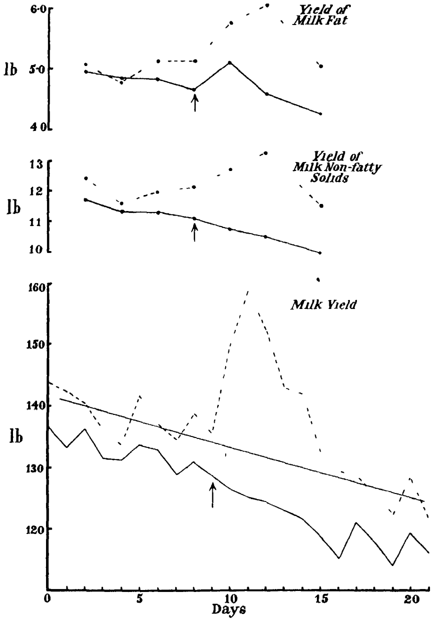

FIG 2 Aggregate yields of milk, milk non fatty solids and milk fat

—— Group A (control cows)

– – – Group B (cows receiving injections of saline extract of anterior pituitary gland)

↑ signifies the last observations before the injections

Milk composition

Curves are shown in figs 1 and 2 which indicate the aggregate amounts of milk fat and milk non-fatty solids secreted by the two groups of cows on particular days during the two experiments It is clear that the injections

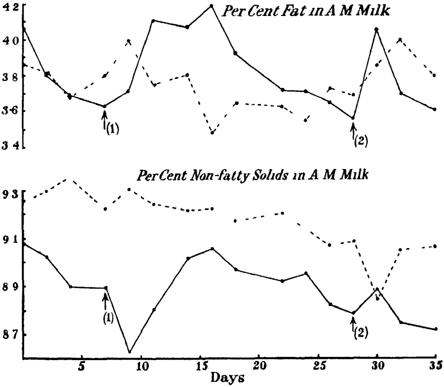

FIG 3 Effect of crude saline extract on percentages of fat and non fatty solids in milk

—— Group A (experimental cows in Experiment 1, control cows in Experiment 2)

– – – Group B (control cows in Experiment 1, experimental cows in Experiment 2)

Non fatty solids percentages corrected for fat volume

↑ (1) signifies the last observations before the injections in Experiment 1

↑ (2) signifies the last observations before the injections in Experiment 2

did exert a real stimulus on milk secretion, as distinct from merely causing dilution of the milk, since the total amounts of fatty and non-fatty solids secreted increased as the result of the injections Fig 3 gives the percentage contents of both types of milk constituent and shows that the injection of the extracts was followed by a transitory lowering of the non-fatty solids content of the milk This temporary decrease was not of sufficient magnitude to cause a similar drop in the total amount of non-fatty solids produced (figs 1 and 2) Since it is probable that the transfer of the water of the milk across the membranes of the mammary gland cells, the synthesis of milk fat and the synthesis of the various components (chiefly caseinogen and lactose) of the milk non-fatty solids are, to some extent, distinct mechanisms, the temporary depression in non-fatty solids content may be explained on the assumption that the response to the injections, of the mechanisms governing water and fat transfer was more rapid than that of those regulating the synthesis of all or part of the non fatty fraction of the milk solids

Determinations of the lactose content of the milk of the individual cows, which were made at regular intervals during Experiment 2, showed that the injections did not cause a decrease in the milk lactose content, on the other hand the caseinogen nitrogen showed a distinct drop on the day following the injections

Though the curves for fat percentage (fig 3) are more variable than those for non-fatty solids, and are therefore more difficult to interpret, it seems that the milk fat content was temporarily increased rather than decreased by the injection This lends support to the view resulting from the work of Graham (1934*a*, *b*) and Folley and White (1936) on the effect of the thyroid gland on lactation, according to which the mechanism regulating the secretion of milk fat is independent of that concerned in the secretion of the non-fatty solids

The increased rate of milk secretion which temporarily followed the injections was not accompanied by any striking alteration in the level of the milk phosphatase

Stimulation of lactation by single injections of prolactin and thyrotropic preparations

(a) Prolactin-C and Thyrotropic-C

For this experiment (Exp 3) two groups of three cows were used Each cow in one group (Group C) received a subcutaneous injection of 30 c c of Prolactin-C, which was equivalent to 10 g fresh anterior lobe tissue, while each cow in the second group (Group D) had an injection of 30 c c of Thyrotropic-C, again equivalent to 10 g fresh tissue

Yield of milk and milk solids The curves for daily milk yield, fat and non-fatty solids, are shown in fig 4 In both groups the injections were quickly followed by a rise in milk yield, although the peak response in the group receiving prolactin was attained a day later than in the group

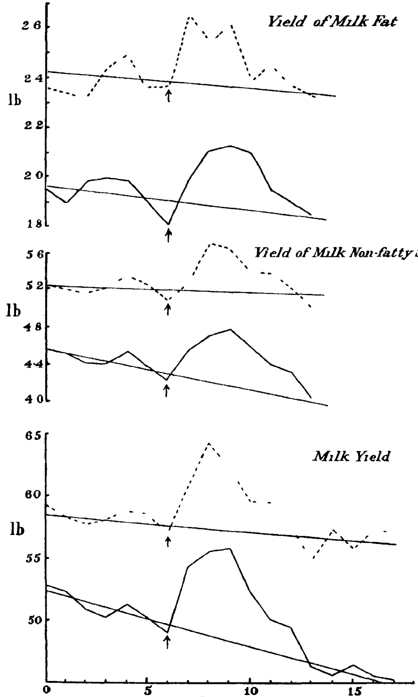
Yield of Milk Fat
2 6
2 4
lb
2 2
2 0
1 8
Yield of Milk Non-fatty Solids
5 6
5 2
lb
4 8
4·4
4 0
Milk Yield
65
60
lb
55
50
0
5
10
15
Days

Fig 4

receiving thyrotropic extract As in the previous experiments the duration of the response and the aggregate "basal" milk yield over the response period for each group were estimated graphically Data for the duration of response in each group and the total increase in yield of milk, milk fat and non-fatty solids are given in Table VII

TABLE VII

	Yield of		
	Milk	Fat	Non fatty solids
Thyrotropic C			
Duration of response, days	5	5	5
Expected yield, lb	287	11 79	25 84
Actual yield, lb	306¼	12 63	27 37
Increase, lb	19¼	0 84	1 53
Total increase as % of "basal" yield	6 7	7 1	5 9
Prolactin C			
Duration of response, days	6	6	6
Expected yield, lb	288¾	11 23	24 88
Actual yield, lb	317¼	12 17	27 33
Increase, lb	28½	0 94	2 45
Total increase as % of "basal" yield	9 9	8 4	9 9

These figures indicate that the response to both extracts lasted for about the same length of time, but the effect of the administration of Prolactin-C on the milk yield was about 25% greater than that of the Thyrotropic C preparation

Milk composition The value for the percentages of fat and non-fatty solids, the latter corrected for fat volume, are plotted in fig 5 The values during the preliminary control period were so variable that it is rather difficult to draw conclusions about the effect of the injections on these two fractions of the milk solids The injections did not appear to be followed by any obvious alterations in the fat percentage With regard to the curves showing alterations in the percentage of milk non fatty solids it will be noticed that the values for both groups of cows show a parallel decline beginning about 3 days before the injections There is some indication, however, that superimposed on this decline is a slight temporary drop in the milk non-fatty solids percentage due to the injections There was no evidence that the injections were followed by any significant change in the milk lactose

FIG 4 Aggregate yields of milk, milk non fatty solids and milk fat

—— Group C (cows receiving injections of Prolactin C)
- - - Group D (cows receiving injections of Thyrotropic C)
↑ signifies the last observations before the injections

content (curves not given) but nitrogen analyses showed that a temporary fall in the milk total nitrogen immediately followed the injections (fig 6) From fig 6 it appears that the decrease in milk total nitrogen following the injections of the prolactin preparation was mainly due to a decrease in caseinogen content, while following the injections of the thyrotropic fraction the decrease in caseinogen, which was of smaller magnitude than in the other case, was accompanied by a decrease in the globulin fraction of the milk proteins The changes in milk phosphatase content during the experimental period were insignificant

(*b*) *Thyrotropic C preparation containing added posterior-lobe extract*

Although the Thyrotropic-C preparation from fresh gland substantially increased the milk secretion of lactating cows, the Thyrotropic 1 preparation

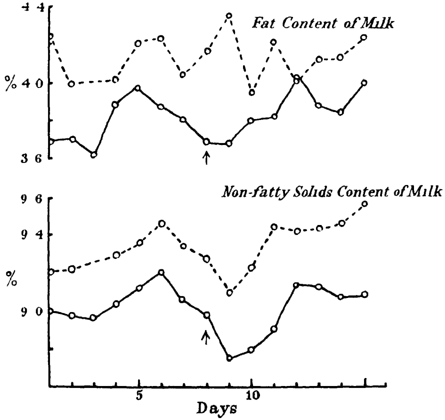

FIG 5 Percentages of fat and non fatty solids in milk
—— Group C (cows given injections of Prolactin C)
– – – Group D (cows given injections of Thyrotropic C)
Non fatty solids percentage corrected for fat volume
↑ signifies the last observations before the injections

from commercial dried gland had, as will be shown below, no effect at all when administered in approximately equivalent amount, and much less effect in larger doses As an important difference between these two thyrotropic preparations was the fact that Thyrotropic 1 possessed sub-

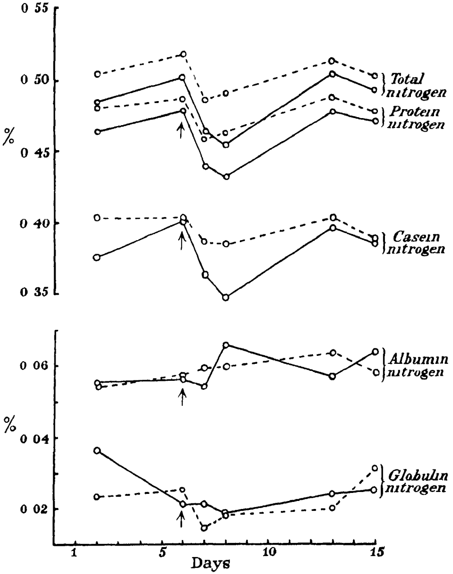

FIG 6 Nitrogen partition of composite samples of A M milk

—— Group C (cows receiving injections of Prolactin C)
- - - Group D (cows receiving injections of Thyrotropic C)

stantial oxytocic activity while Thyrotropic C possessed virtually none, it seemed necessary to determine whether or not the addition of posterior lobe extract to the Thyrotropic-C preparation would inhibit its lactogenic activity Accordingly an experiment (Exp 4) was carried out in which three cows in one group (E) each received a single injection of 30 c c of Thyrotropic-C as before, while those of another similar group (F) received a similar amount of Thyrotropic-C preparation to which had been added 2 c c of a posterior lobe extract containing approximately 125 international units of oxytocic activity

The experimental procedure was, in general, similar to that adopted in Exp 3

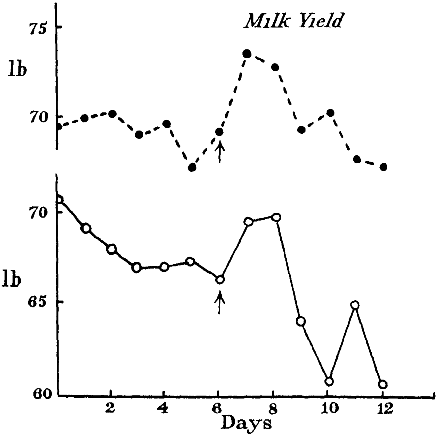

FIG 7 Aggregate milk yields

●---● Group E (cows receiving injections of Thyrotropic C)

○——○ Group F (cows receiving injections of Thyrotropic C+posterior lobe extract)

↑ signifies the last observations before the injections

The mean daily milk yields for both groups are plotted in fig 7 from which it is evident that the lactogenic effect of single injections of Thyrotropic-C is not inhibited by the presence of posterior lobe extract

(c) *Thyrotropic* 1 *preparation*

For this experiment (Exp 5) two groups of three cows were used

After the usual preliminary control period each cow of Group G received one injection of 250 mg of Thyrotropic 1 dissolved in 30 c c water, while at the same time each cow of Group H was given an injection of 500 mg in the same volume of water Reference to Table II will show that on the basis of the Rowlands and Parkes (1934) method of assay of thyrotropic activity, 250 mg of Thyrotropic 1 preparation is definitely more active than 30 c c of Thyrotropic-C Curves showing for each group the total daily milk yield, total daily yield of milk non-fatty solids and percentages of fat and non-fatty solids in each day's milk are given in fig 8 Injection of the higher dose of Thyrotropic 1 was followed by a slight increase in milk yield, a rise in the daily production of milk non fatty solids and a striking increase in the concentration of the latter in the milk The milk lactose content underwent no striking alterations as a result of the injections at either dosage (curves not given) The fat percentage was rather variable and the injections caused no obvious changes during the period of observations The curves for the cows on the lower dosage show very slight indications of similar changes but in no case could the effect be considered significant In the case of the curves showing the changes in the yield of milk and milk non-fatty solids due to the injection of 500 mg , the basal yield and the percentage increases due to the treatment were estimated as in previous cases from lines of best fit, which are shown in fig 8 The values so obtained are given in Table VIII

TABLE VIII

	Duration of response days	Expected yield lb	Actual yield lb	Increase lb	Total increase as % of "basal" yield
Yield of milk	4	304	315¼	11¼	3 7
Yield of milk non fatty solids	4	27 54	28 89	1 35	4 9

A comparison of these results with those in Table VII shows that the lactogenic activities of Thyrotropic 1 and Thyrotropic-C are by no means proportional to their respective thyrotropic activities, assayed by ability to increase the weight of the thyroid gland (Table II), since a single dose of Thyrotropic 1, more than equal in thyrotropic activity to 30 c c

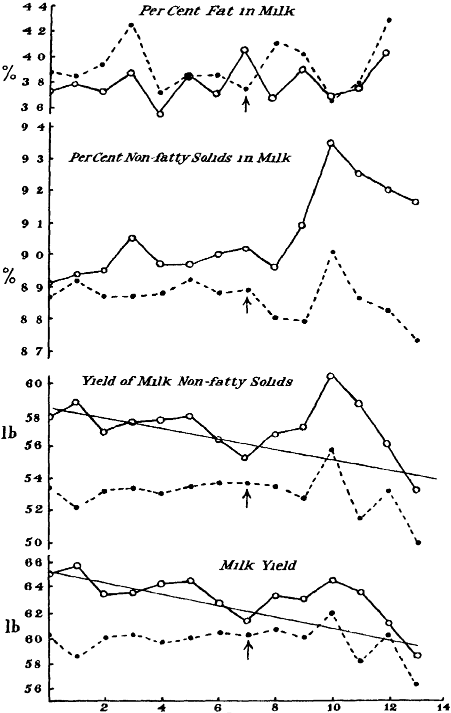
Per Cent Fat in Milk
4 4
4 2
4 0
3 8
3 6
%
Per Cent Non-fatty Solids in Milk
9 4
9 3
9 2
9 1
9 0
8 9
8 8
8 7
%
Yield of Milk Non-fatty Solids
60
58
56
54
52
50
lb
Milk Yield
66
64
62
60
58
56
lb
2
4
6
8
10
12
14
Days

FIG 8

Thyrotropic-C, showed no significant lactogenic activity, while double that dose evoked a distinctly smaller lactogenic response than that following an injection of 30 c c of Thyrotropic-C This point will be discussed below

(*d*) *Prolactin* 1 *preparation*

Each of three cows received a subcutaneous injection of 200 mg of Prolactin 1 preparation dissolved in 30 c c water containing NaOH-NaCl at *p*H 8 0 (Exp 6) The pigeon crop stimulating activity of this dose was about twice that of 30 c c of Prolactin-C

The curves for milk yield, fat percentage and milk non-fatty solids percentage which are plotted in fig 9 show that the single injection of 200 mg of Prolactin 1 caused no stimulation of milk secretion, nor any significant alteration in milk composition The milk lactose content remained practically constant during the experiment (curves not given) The result of this experiment is in contrast to that of Exp 3, in which a single dose of Prolactin C containing about half the crop stimulating activity present in 200 mg of Prolactin 1 caused a marked stimulation of milk secretion

Stimulation of lactation by repeated injections of Prolactin preparations 1 *and* 2

Two experiments (7 and 8) come under this category, each involving two cows, one experimental and one control The considerable expense associated with the preparation of purified prolactin prevented the use of groups of cows for these experiments One cow was given fifteen daily subcutaneous injections of 1 g of Prolactin 1 dissolved in 50 c c NaOH-NaCl at *p*H 8 0 The control animal received similar injections of distilled water Another cow received seven daily subcutaneous injections of 0 5 g of Prolactin 2 in 50 c c NaOH-NaCl at *p*H 8 0 On the eighth day the daily dose of Prolactin 2 was increased to 0 75 g dissolved in 75 c c of NaOH-NaCl at *p*H 8 0 and the injections continued thus for a total of 6 days Control injections of distilled water were given simultaneously to another cow

Milk yield Mean daily milk yields over successive 3-day periods for each cow are plotted in fig 10 In both cases the injections caused an

FIG 8 Aggregate yields of milk and non fatty solids and percentages of fat and non fatty solids in milk

●- - -● Group G (cows receiving injections of 250 mg Thyrotropic 1 preparation)

○- - -○ Group H (cows receiving injections of 500 mg Thyrotropic 1 preparation)

↑ signifies the last observations before the injections

increase in milk yield In the first experiment (No 7) it is seen that throughout the injection period the milk yield of the experimental cow rose steadily to a peak which was reached soon after the injections ceased It is seen from fig 10 that the response lasted for at least a month after the cessation

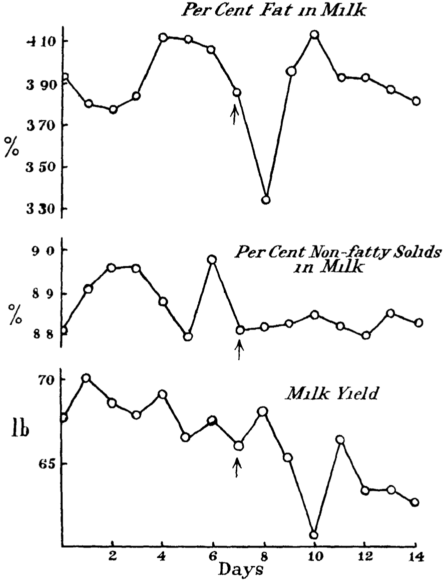

Fig 9 Yields of milk and percentages of fat and non fatty solids in milk relating to a group of 3 cows each receiving a single injection of Prolactin 1

↑ signifies the last observations before the injections

of injections, probably much longer, and it seems that injections of Prolactin 1 had the effect of raising the lactation curve to a level higher than normal, from which the usual decline set in at about the normal rate

In Exp 8 (Prolactin 2) the daily dose of prolactin was less and the increase in milk yield above "basal" was not so marked, and disappeared within 3–4 days of the cessation of the injections (fig 10)

Milk composition Curves showing values for milk fat and non-fatty solids contents for Exps 7 and 8 are shown in fig 11 There was no fall in the level of either type of milk constituent, nor was there a rise in milk fat content, though there is some evidence that treatment with prolactin at the higher level of dosage increased the non fatty solids content of the milk

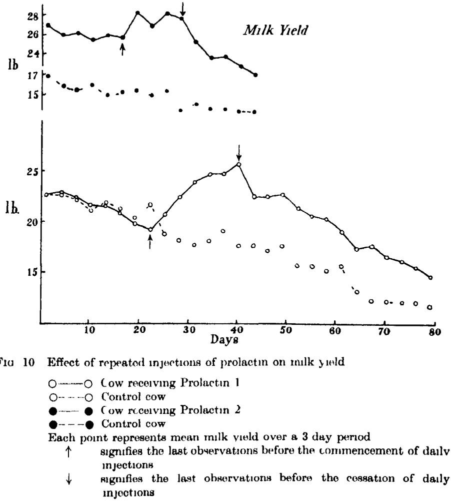

FIG 10 Effect of repeated injections of prolactin on milk yield

○——○ Cow receiving Prolactin 1
○– – –○ Control cow
●—— ● Cow receiving Prolactin 2
●– – –● Control cow

Each point represents mean milk yield over a 3 day period

↑ signifies the last observations before the commencement of daily injections

↓ signifies the last observations before the cessation of daily injections

The curves showing variations in milk lactose and chlorides in Exps 7 and 8 (fig 12) are of great interest, since in both experiments the lactose content of the milk of the experimental animal increased during the injection period, the controls showing no such effect Variations in the concentration of milk lactose are usually accompanied by reverse variations in the chloride content (Mathieu and Ferre 1914), and in both experiments the milk showed a decrease in chloride content during the injection period No significant alterations in the milk phosphatase level during the injection period were observed in either experiment (curves not given)

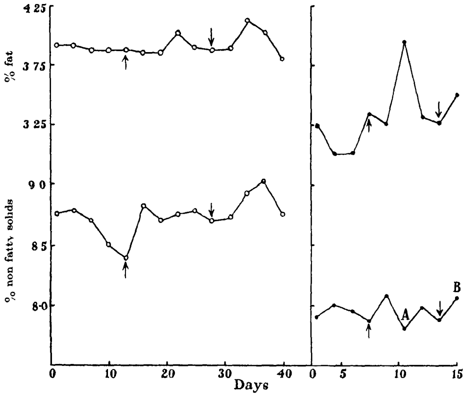

FIG 11 Mean concentration of fat and non fatty solids over 3 day periods in milks of cows receiving daily injections of Prolactin 1

○— —○ Cow receiving Prolactin 1
●——● Control cow
↑ signifies the last observations before the commencement of daily injections
↓ signifies the last observations before the cessation of daily injections
A Two day period (sample lost) B Two day period

Blood sugar The results of true blood-sugar determinations carried out during Exp 7 indicated that there was no significant alteration in the blood-sugar level during the injection period

Discussion

The results of our experiments with a crude saline extract of fresh anterior lobe tissue are in general agreement with those of Azimov and Krouze (1937) who used an alkaline extract of anterior pituitary gland It

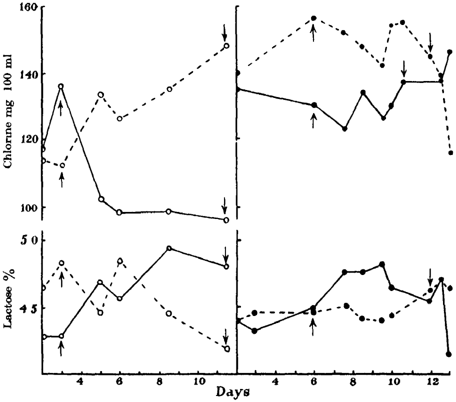

FIG 12 Effect of daily injections of prolactin on the percentages of lactose and chlorine in A M milk

○ ——○ Cow receiving Prolactin 1
○- - -○ Control cow
● ——● Cow receiving Prolactin 2
●- - -● Control cow
↑ signifies the last observations before the commencement of daily injections
↓ signifies the last observations before the cessation of daily injections

seems quite clearly demonstrated that a single injection of such crude extracts into cows in the declining phase of the lactation cycle can cause a substantial but temporary increase in milk volume It should be mentioned that in some instances the subcutaneous administration of our crude saline extract caused lumps to develop at the site of injection These did not seem to be painful, however, and usually disappeared within a week without treatment None of the other pituitary preparations used in the present investigations caused any obvious reaction at the site of injection In our experiments a single injection of crude extract equivalent to 5 g of fresh anterior lobe appeared to exert a maximal effect on lactation, since the injection of double that amount of extract had no greater effect

Our results differ in one point of detail from those of Azimov and Krouze The Russian workers found that the administration of their extract caused no observable alteration in milk composition, while we found that a temporary slight fall in the nitrogenous non-fatty solids frequently followed the administration of the crude extract

The Thyrotropic-C and Prolactin-C preparations exerted similar effects, causing a substantial temporary increase in milk volume This result is particularly interesting as the Thyrotropic-C preparation contained no detectable amount of the substance that stimulates growth of the pigeon crop-gland (prolactin), which suggests that anterior lobe hormones other than prolactin possess the power of stimulating milk secretion in cows in declining lactation This conclusion receives strong support from the result of the experiment in which a single injection of Prolactin 1 failed to influence the milk volume, although the amount of this preparation used possessed twice the pigeon crop-gland stimulating activity of that dose of Prolactin-C which had previously been found, in similar conditions, to exert a marked lactogenic effect

The daily administration of a large dose of Prolactin 1 to a cow (Exp 7) induced a substantial and prolonged increase in the milk yield, the lactose content being slightly raised, although the blood-sugar level was not affected This type of treatment may have exerted a more lasting effect on the mammary gland than the transitory effects which have been found to result from single injections of the pituitary preparations The latter type of effect is such as would follow a temporary improvement of the supply of the milk precursors which are brought to the mammary gland in the blood, while the former seems to be associated with a permanent (i e permanent as far as the lactation in question is concerned) improvement in the functional efficiency of the mammary gland It is probable that the normal decline in the rate of milk secretion is due, partially at any rate, to the gradual involution of the lobule-alveolar system of the mammary gland and there is a possibility that repeated injections of the prolactin preparation induced a partial regeneration of the involuted tissue If this were true, it should be possible by such treatment greatly to increase the lactation period of non-pregnant cows Obviously, further work on this important aspect of the problem is desirable and such is contemplated It may be mentioned that the daily administration of a prolactin preparation of the type of Prolactin 1 can induce a copious and prolonged secretion of milk in rabbits (cf Young 1938*c*), the composition of the milk being normal for this animal (Folley and Young, unpublished data)

TABLE IX SUMMARY OF THE BIOLOGICAL PROPERTIES OF THE EXTRACTS USED IN THE PRESENT INVESTIGATION

Starting material	Extract	Pigeon crop gland stimulating activity	Lactogenic activity (single injection)	Glyco tropic activity	Diabeto genic activity	Thyro-tropic activity
Fresh ox anterior lobe	Crude saline extract	+ + +	+ + +	+ + +	+ + +	+ + +
	Prolactin C	+ +	+ + +	+ + +	0	Trace
	Thyrotropic C	0	+ +	+ +	+ +	+ + +
Commercial dried ox anterior lobe	Prolactin 1	+ +	0 ?*	+	0	0
	Thyrotropic 1	0	+	+ ?	0	+ +

* No effect when a single injection was given Substantial effect when daily injections of a large dose were given over a period

Table IX† gives a summary of the biological properties of the different preparations used in the present investigation It is clear that the pigeon crop-gland stimulating activities of these extracts do not parallel their lactogenic activities on cows in declining lactation On the other hand, there does seem to be a surprisingly close parallelism between the lactogenic activities of these extracts and their glycotropic activities (see Table X) We are not justified, on this basis, in assuming that the glycotropic factor is a "lactation hormone", since the mechanism by which anterior pituitary extracts increase the rate of milk secretion must remain, at the present time, largely a matter of speculation Lactation is a complex physiological phenomenon governed by many factors, any one of which may be a limiting one in a particular case There is considerable evidence that the lactating mammary gland absorbs large amounts of sugar from the blood (see Blackwood and Stirling 1932, Lintzel 1934 Graham, Jones and Kay 1936, Graham 1937) which it may partly transform into lactose (see, for example, Grant 1935) and partly metabolize There is good reason for the belief that the blood-sugar level may be one of the limiting factors in lactation (see Graham, Jones and Kay 1936), a fall of blood sugar tending to decrease milk-secretion (Gowen and Tobey 1931*a*, *b*) As the action of the glycotropic factor antagonizes any tendency to hypoglycaemia (Young 1936*b*) the injection of a pituitary preparation possessing glycotropic activity might be expected to exert a favourable influence on lactation by protecting the blood-sugar level against the large demands made upon it by the lactating mammary gland There is therefore no difficulty in assuming that, in the cow in declining lactation, the glycotropic factor of the anterior pituitary lobe might constitute one of the limiting factors controlling the amount of milk secreted

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† In this the degree of activity is indicated by plus signs, one for some activity, two for a moderate activity and three for high activity

TABLE X COMPARISON OF THE GLYCOTROPIC ACTIVITIES AND LACTOGENIC ACTIVITIES, AS ASSESSED BY THE RESULTS OF SINGLE INJECTIONS INTO COWS IN DECLINING LACTATION, OF THE EXTRACTS USED IN THIS INVESTIGATION

Extract	Number of grams of fresh tissue equivalent to the dose extract given in single injection	Increase in milk yield as % of "basal" yield	Amount of extract required to abolish or nearly to abolish the hypoglycaemic action of insulin in the normal rabbit under the conditions described
Crude saline	5	10 3	4 c c
Crude saline	10	11 6	4 c c
Prolactin C	10	9 9	6 c c
Thyrotropic C	10	6 7	9 c c
Thyrotropic I	205	3 7	150–200 mg (?)*
Prolactin I	15	0 0	100 mg

* See Table III

It seems that at present there is no satisfactory evidence that the pituitary gland secretes a single ' lactation hormone" "Prolactin" (the anterior pituitary substance which stimulates growth of the pigeon crop-gland) is certainly not the only lactogenic substance in the hypophysis It therefore seems to us that use of the term "prolactin" should be confined to describing the anterior lobe substance characterized by capacity to promote growth of the pigeon crop-gland, without prejudice as to whether or not it is also necessarily lactogenic Those extracts which stimulate the secretion of *normal* milk in the mammal should be described as containing a lactogenic substance or substances

The relative lactogenic potencies of the two thyrotropic preparations (Thyrotropic 1 and Thyrotropic-C) were very different from their respective thyrotropic activities and it seemed more probable that the lactogenic properties of these extracts were correlated with their respective contents of glycotropic factor than with their thyrotropic activities It is, however, possible that the lactogenic effect of these two thyrotropic extracts was indeed partly due to their ability to stimulate the secretion of thyroid hormone, although the method of assay of thyrotropic activity, employing the growth of the guinea-pig thyroid gland, is an unsatisfactory criterion of the power of an extract to promote the secretion of the thyroid hormone (see Heyl and Laqueur 1935) At present no definite conclusion can be drawn upon this point

The extra milk secreted under the influence of the pituitary extracts was of normal composition The slight rise in milk-fat and lactose content, and the slight fall in content of nitrogenous non fatty solids, which occurred in some instances, probably fall within the limits of normal variation in milk composition The comparatively insignificant nature of the alterations in the milk phosphatase content observed throughout these experiments is somewhat surprising in view of previous findings which showed that natural and experimental alterations in the functional efficiency of the mammary gland are accompanied by significant changes in the milk phosphatase content (see Folley and White 1936 Folley and Kay 1936*b*, Folley 1936) No explanation for this can be offered at present

In conclusion it is perhaps not out of place, in addition to emphasizing the possible clinical importance of the experimental stimulation of milk yield by the administration of pituitary extracts, briefly to consider the financial aspects of the problem as applied to dairy farming Azimov and Krouze (1937) stated that their experiments resulted in a financial profit The cost of the specially frozen ox pituitary glands, used in our experiments to prepare 15 c c of the crude saline extract, or alternatively 15 c c each of Prolactin-C and Thyrotropic C, was 1*s* 6*d* The extra milk secreted under the influence of the injection of 15 c c of the crude saline extract into each of six cows could be sold for approximately 12*s*, which is about 33 % more than the cost of the glands used It is possible to obtain frozen ox pituitary glands which have been less carefully treated for considerably less than the price of the glands we used It might be possible to devise a method for preparing an extract approximately equivalent to our Thyrotropic-C, which would require comparatively little labour, of a relatively unskilled type, making use of a cream-separator as a centrifuge If results comparable with ours were obtained under these conditions, the possibility that such methods might be employed by farmers, particularly in times of food shortage, would merit serious consideration

We are greatly indebted to Professor R Rae and Mr K W D Campbell of the University of Reading Farm, Sonning, for their co-operation, and to Mr J Mackintosh for access to and management of the Shinfield cows We also wish to thank Dr I W Rowlands for a number of prolactin and thyrotropic assays, and Dr W L Davies for determinations of milk nitrogen partition

SUMMARY

1 The possibility of increasing the milk yield of lactating cows in declining lactation by single injections of the following ox anterior pituitary extracts has been investigated a crude saline extract of fresh gland, prolactin and thyrotropic fractions prepared at low temperature from fresh gland, prolactin and thyrotropic fractions prepared at room temperature from commercial, acetone-desiccated anterior lobe tissue The chief difference between the preparations obtained at low temperature from the fresh gland and those prepared at normal temperature from the dried tissue is that the former possess much greater glycotropic (anti-insulin) activity than do the latter

2 Single injections of the crude saline extract, and of the prolactin and thyrotropic preparations obtained from fresh gland resulted, in each case, in a substantial increase in the milk yield for 5 or 6 days Only slight changes were found in the composition of the milk

3 A single injection of prolactin prepared from dried gland administered in a dose equivalent, as regards crop-gland stimulation, to the dose of prolactin from the fresh gland, had no effect on the milk volume or composition Likewise a single injection of a dose of the thyrotropic fraction prepared from dried gland, more than equivalent to the dose previously used of the thyrotropic fraction from fresh gland as regards ability to cause enlargement of the thyroid gland, had no effect on the milk volume or composition, though double that dose did cause a temporary increase in milk yield accompanied by an increase in the milk non-fatty solids content

4 The daily injection of very large doses of prolactin prepared from dried gland resulted in a substantial and prolonged increase in milk volume, with a rise in milk lactose content accompanied by a fall in the milk chlorides

5 The thyrotropic preparation from fresh gland, although lactogenic in the lactating cow, possessed no detectable pigeon crop-gland stimulating activity The prolactin preparation from dried gland, although possessing substantial pigeon crop-gland stimulating activity, failed to exhibit lactogenic activity when a single dose was injected into a lactating cow The relative lactogenic activities of the extracts used are paralleled, not by their activities in causing stimulation of the pigeon crop gland, but rather by their relative glycotropic activities

6 There seems to be no satisfactory evidence that a single "lactation hormone" is secreted by the anterior pituitary gland, and it is suggested that use of this term should be discontinued

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Selective neutralization of the luteinizing activity of gonadotrophic extracts of pituitary by anti-sera

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(*Communicated by A S Parkes, F R S —Received* 16 *May* 1938)

[Plates 1-3]

Gonadotrophic extracts from different sources vary greatly in their relative capacity to cause follicular stimulation, ovulation and luteinization in the ovaries of the immature rat Thus the effectiveness of crude extracts of anterior pituitary depends largely on the species of animal from which the pituitaries are obtained (Hill 1934) Extracts of horse pituitary cause great activity in the immature ovaries, leading to the production of abundant large follicles and corpora lutea with or without ovulation Extracts of sheep and pig pituitaries are much less effective, while extracts of ox pituitary show little activity when tested on the immature rat Extracts from urine of pregnancy cause some growth of the follicles, which tend to become heavily luteinized, but those of urine from women after the menopause and from ovariectomized women show greater follicle-stimulating properties (Zondek 1930)

The differences between these various types of extract are accentuated when the test animal is hypophysectomized Pituitary extracts usually produce similar effects in the ovaries of normal and hypophysectomized rats (Smith 1930), extracts of urine of pregnancy cause extensive luteinization of the interstitial and thecal tissue but no follicular development in the ovaries of hypophysectomized rats (Selye, Collip and Thomson 1933, Leonard and Smith 1934) On the other hand, extracts of urine from women after the menopause produce follicle stimulation without luteinization (Leonard and Smith 1933, Tyndale and Levin 1937) These differences in the qualitative activity of gonadotrophic extracts are most easily explained by assuming that two gonadotrophic hormones occur in the pituitary, one causing growth and maturation of the follicle (follicle-stimulating hormone- F S H) and the other luteinization (luteinizing hormone—L H), and that they occur in different proportions in extracts from different sources A separation of these two substances from a crude pyridine extract of sheep pituitary was reported by Fevold, Hisaw and Leonard (1931), who made use of the fact that the two hormones were not equally soluble in water They have since (Fevold and Hisaw 1934, Fevold 1937) recorded modifications of technique and given details of results Consideration of the pituitary gonadotrophic complex is complicated by evidence which suggests the presence of antagonistic, synergistic and interstitial cell stimulating substances (Evans and others 1936) which have to be considered in relation to the biological properties of anti gonadotrophic sera (see Rowlands 1938, for instance)

Since extracts from different sources differ qualitatively it is likely that the anti-gonadotrophic sera raised against them will also differ qualitatively, quite apart from any immunological species specificity Thus an extract predominantly containing F S H might be expected to evoke an anti serum which would mainly inhibit follicle stimulation, while an extract with an excess of L H would produce one having a greater anti-luteinizing action The reaction between any particular extract and anti-serum would then depend on the relative amounts of F S H and L H in the extract and anti-F S H and anti-L H in the anti serum Thus it should be possible by combination of appropriate extract and anti serum to neutralize completely either the F S H or the L H leaving most of the other free, thereby considerably altering the qualitative activity of the extract Since extracts of horse pituitary seem to contain much F S H and relatively little L H, whereas extracts of ox pituitary seem to contain relatively much L H and little F S H, a promising combination to produce a preparation with purely follicle-stimulating properties would seem to be horse pituitary extract plus anti serum to ox pituitary A previous attempt (Rowlands 1937) to achieve a differential neutralization of the luteinizing effect by such a combination was unsuccessful Complete neutralization of the L H required an amount of anti-serum sufficient to neutralize nearly all the F S H

More recent work has made it possible to obtain, under certain apparently reproducible conditions, anti-sera to ox pituitary extracts which will neutralize the power of horse pituitary extracts to luteinize the membrana granulosa, but which leave in active form the greater part, if not all, of the follicle stimulating activity

METHODS

Preparation of extracts Two gonadotrophic extracts of horse pituitary were used

(1) Pituitary glands from mares and geldings were desiccated in acetone, ground and kept for 12 hr at 0° C with 15 vol of $N/20$ NaOH The mixture was neutralized and centrifuged, and the supernatant extract precipitated with 5 vol alcohol and 1 vol ether The residue was twice re-extracted by the same procedure The three extracts were mixed, a total of 6 75 g of dry powder (AP 2 B) being obtained from 14 2 g of desiccated gland

(2) 63 g of acetone-dried gelding pituitaries were extracted with 10 vol of water at pH 8 The residue was re extracted with a similar volume at pH 10, and again with 10 vol of $N/30$ NaOH The combined extracts were precipitated by the addition of 6 vol of alcohol, and the precipitate collected and dried (AP 70 B) 17 6 g of extract were obtained

The activity of these extracts is shown in Table I

Production of anti-sera (1) A group of rabbits (ATH 23–69) was injected daily with 25 mg of a pyridine extract of ox pituitary, as described by Rowlands and Parkes (1936), and bled from the carotid artery under ether anaesthesia after a period of about 5 months The serum was fractionated (Harington and Rowlands 1937, Exp 6) and the globulin obtained as a white soluble powder, of which 34 mg represented 1 c c of original serum (Serum Prep 6)

(3) Another batch of ten rabbits (ATH 104–113) was injected daily with a similar extract until bled after a period of 3 months The serum was obtained in the usual way No attempt was made to fractionate this serum (Serum A)

Biological tests The horse pituitary extracts were tested by their ability to stimulate ovarian enlargement in the immature rat weighing 40–45 g In untreated rats of this body weight the ovaries weigh 10–12 mg and the uterus 25–30 mg Groups of five to ten rats were injected once daily for

5 days and killed 24 hr after the last injection The ovaries and uterus were dissected and fixed overnight in Bouin s fluid After immersion in 70% alcohol they were weighed on a torsion balance

TABLE I THE EFFECTS OF ANTI-SERA TO GONADOTROPHIC EXTRACTS OF OX PITUITARY IN NEUTRALIZING THE LUTEINIZING ACTIVITY OF EXTRACT OF HORSE PITUITARY

Extract		Serum		Weight of		Proportion of rats showing luteinization of membrana granulosa
Description	Amount mg	Description	Amount c c	Ovaries mg	Uterus mg	
AP 2 B	10	-	—	74	69	10/10
		Prep 6	≡ 2 50	72	115	0/10
		Serum A	2 50	67	119	0/5
AP 70 B	2 5	—		54	65	5/5
		Normal	1 25	68	88	4/5
		,,	2 50	76	78	5/5
		,,	5 00	76	71	5/5
		Serum A	0 50	73	70	5/5
		,	1 00	87	82	2/5
		,	1 25	46	110	0/10
		,,	1 50	24	105	0/10
			2 00	20	98	0/10
		,	3 00	15	89	0/5
			5 00	11	52	0/5

Serial sections of both ovaries of all rats were cut at 10μ and stained in Meyer's haemalum and eosin Lipschutz (1934) has described a method of estimating the amount of luteinization taking place, but it is not very convenient for large scale work In evaluating the results recorded below the proportion of animals in a group showing any luteinization of the membrana granulosa has been taken as an index of the degree of luteinization, and the percentage showing no luteinization as the index of pure follicle stimulation

RESULTS

The various results obtained are given in Tables I and II

(*a*) *Extract AP2B given alone* A total dose of 10 mg of the extract AP2B injected into a group of ten immature rats produced an average ovarian weight of 74 mg , each ovary containing large numbers of corpora lutea (fig 1, Plate 1) The average weight of the uteri was 69 mg , they were in the condition seen during the luteal phase of the oestrous cycle (*b*) *Extract AP 2 B with serum Prep* 6 The simultaneous injection of an equivalent of 2 5 c c of the ox pituitary anti-serum (Prep 6) with 10 mg of extract AP2B did not significantly alter the ovarian weight response resulting from the injection of the same amount of extract alone But macroscopically and histologically (see fig 2, Plate 1), however, the response of the ovary was completely changed No corpora lutea were present, the ovaries contained only numerous follicles in various stages of maturation, many being fully developed No luteinization of the membrana granulosa had occurred The uteri were very large and distended with fluid such as occurs at oestrus in the normal animal, their weight was nearly twice as great as that of the uteri of the control animals receiving only the extract

(*c*) *Extract AP 2 B with serum A* When 10 mg of the extract were given in conjunction with 2 5 c c of serum A a similar inhibition of the luteinizing action of the extract was produced The average weight of ovary was somewhat less than in the control group, but histologically, all the ovaries contained large unruptured follicles (fig 3, Plate 1) The uteri, as before, were greatly distended with fluid

(*d*) *Extract AP 70 B given alone* A dose/response curve was constructed for this extract given alone (fig 9, Table II) On the basis of ovarian enlargement the extract is nearly four times as active per mg as AP2B, a total dose of 2 5 mg giving an average ovary weight of 54 mg These ovaries all contained corpora lutea (fig 4, Plate 2) Smaller or larger doses than this show a typical dose/response relation From the present standpoint it is important to notice that the dose has to be lowered to a point where the ovarian weight is only 29 mg before the number of animals showing luteinization is decreased

A large quantity of the extract was available and it was possible to carry out detailed experiments

(*e*) *Extract AP 70 B with normal serum* An investigation was first made of the effect of giving different amounts of normal rabbit serum with a constant dose (2 5 mg) of the extract (fig 10) No effect was observed except slight augmentation in ovary weight due probably to alteration of the conditions of the absorption of the extract by the simultaneous introduction of a large amount of foreign protein Luteinization (fig 5, Plate 2) occurred in the ovaries of all the rats except one, which received 1 25 c c of normal serum, and which showed a particularly poor degree of ovarian stimulation

(*f*) *Extract AP70B with serum A* Very different results were obtained with this combination (fig 11) The administration of a total of 0 5 c c of

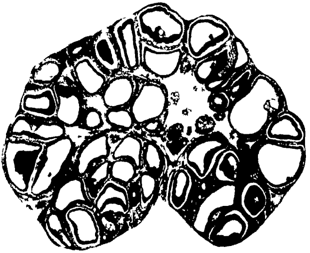

FIG 3

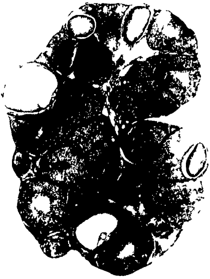

FIG 1

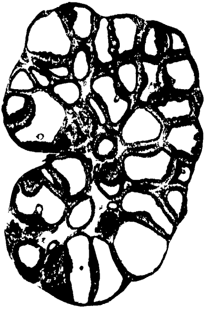

FIG 2

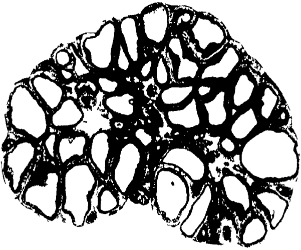

Fig 6

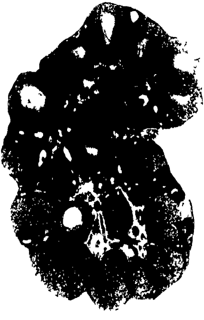

Fig 4

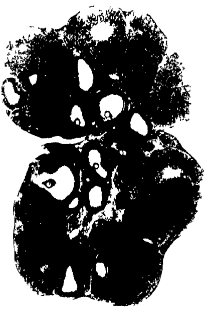

Fig 5

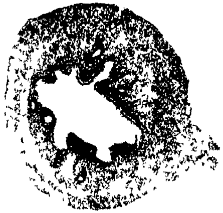

Fig. 7

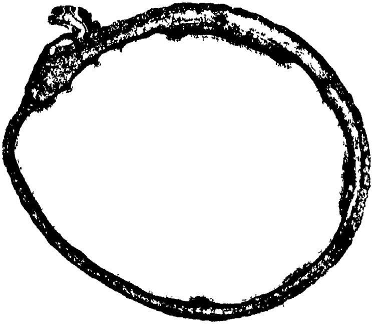

Fig. 8

serum A with 2·5 mg. of the extract caused an augmentation in the effect on the weight of the ovaries. A total of 1·0 c.c. of serum A gave a similar but more marked result. This augmentation is greater than that produced by similar amounts of normal serum and is probably due to the same causes as those previously described (Rowlands 1938) in connexion with anti-sera to sheep and pig pituitary extracts. Abundant corpora lutea were found in each of the rats receiving 0·5 c.c. of serum A in addition to the 2·5 mg. AP70B, and the uteri were of the luteal type. In those receiving 1·0 c.c. of the serum, however, differential neutralization of the L.H. had begun and in spite of the large size of the ovaries, only two of the five showed luteinization of the membrana granulosa. The injection of 1·25 c.c. of serum A with 2·5 mg. of AP70B caused a slight decrease in the ovarian weight response compared with that produced by the extract alone, but luteinization of the membrana granulosa was completely inhibited in all the rats (fig. 6, Plate 2). Numerous follicles of various sizes, many mature, were present. The theca interna in some of the large follicles was slightly thicker than is usual at oestrus. This was due to a slight increase in the size of the individual cells, which occasionally appeared to be slightly luteinized. The uteri in this group were extremely distended (fig. 8, Plate 3) and showed maximal oestrous stimulation.

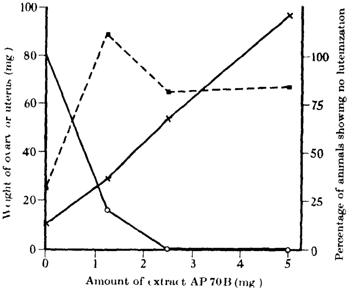

FIG. 9. Dose/response curve for effect of horse pituitary extract AP70B, on immature female rats. × Ovary weight. ■ Uterus weight. ○ Percentage of rats showing no luteinization.

Increase in the amount of serum led to progressive decrease in the ovarian weight response to 2·5 mg. AP70B, and to decrease in the number and size of the follicles present. No corpora lutea or luteinization of the membrana granulosa were present in any of the rats receiving more than a total of 1·0 c.c. of serum. The amount of anti-serum required to inhibit completely the weight increase in the ovary is about four times that required to effect the maximum qualitative modification in the response, i.e. about 5 c.c. Even with this high dose of serum, enlargement of the uteri occurs indicating stimulation of the endocrine activity of the ovary after the weight response is inhibited. Microscopic examination showed that in all these ovaries one or two large follicles were present which were apparently insufficient to cause any detectable increase in ovarian weight.

Comparison of figs. 9 and 11 shows definitely that the effect produced by the simultaneous administration of 2·5 mg. of AP70B and 1·25 c.c. of serum A is not similar to that produced by giving smaller doses of AP70B alone, and is not due therefore merely to the partial quantitative neutralization of the extract by the serum.

(*g*) *Dose/response relation for the optimum combination of AP70B and serum* A. The combination of extract and serum which completely altered the qualitative nature of the response without appreciably reducing the increase in the resulting weight of the ovary was 2·5 mg. plus 1·25 c.c. serum, or 2 mg. of extract to 1 c.c. of serum. This particular proportion was therefore considered optimal and an attempt was made to examine the effect of varying the total amount given, keeping the proportion constant. The results are shown in Table II in comparison with those obtained for corresponding amounts of AP70B alone.

When small amounts of serum and extract (1·25 mg. + 0·625 c.c.) were given, the response measured by weight of ovary, was greater than that given by the group receiving the same amount of extract alone. Histologically, the ovaries were found to contain only mature follicles, luteinization having been inhibited. The injection of four times this amount of both serum and extract, however, caused the production of very large ovaries which were characterized by follicular stimulation to a much greater extent than those of the rats receiving 5·0 mg. of extract alone. In four of the five rats, however, several of the large follicles showed some luteinization of the membrana granulosa and the luteinization index has therefore to be given as 4/5, though this does not reflect the extent of the effect of the serum at this dose level. Some luteinization of the theca interna, and of the interstitial tissue had occurred in all the rats receiving 5 mg. of extract and 2·5 c.c. of serum.

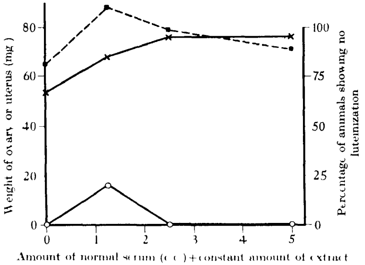

Fig 10 The effect of varying amounts of normal rabbit serum on the response caused by a constant amount of pituitary extract (AP70B) × Weight of ovary ■ Weight of uterus ○ Percentage of rats showing no luteinization

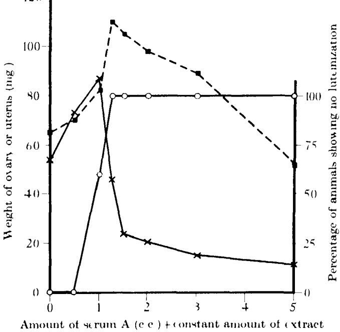

Fig 11 The effect of varying amounts of serum A (anti serum to ox pituitary) on the response caused by a constant amount of pituitary extract (AP70B) × Weight of ovary ■ Weight of uterus ○ Percentage of rats showing no luteinization

The uteri of the rats receiving 1.25 or 2.5 mg. of extract plus serum showed full oestrous distension, but not in those receiving 5.0 mg. plus serum, a result easily correlated with the histological findings in the ovary.

TABLE II. THE EFFECT OF DIFFERENT AMOUNTS OF EXTRACT AP70B AND SERUM A IN A CONSTANT RATIO, ON THE QUANTITATIVE AND QUALITATIVE RESPONSE IN THE RAT OVARY

Amount of extract mg.	Amount of serum c.c.	Weight of Ovary mg.	Weight of Uterus mg.	Proportion of rats showing luteinization of membrana granulosa
1.25	—	29	89	4/5
	0.625	46	137	0/5
2.50	—	54	65	5/5
	1.250	46	110	0/5
5.00	—	97	67	5/5
	2.500	104	89	4/5

DISCUSSION

The results described above show clearly that the qualitative activity of a gonadotrophic extract can be completely changed by the simultaneous injection of a particular type of anti-serum. The most simple explanation of the results so far obtained is to suppose that the luteinizing substance in extracts of horse pituitary is differentially neutralized by anti-ox pituitary serum, which is mainly anti luteinizing in effect. The residual activity of the horse pituitary extract is mainly or exclusively follicle-stimulating, and if the balance between extract and anti-serum is carefully adjusted, it is sufficiently great to produce considerable growth of follicles in the ovary of the immature rat.

It should be remembered, however, that the activity of the animal's own pituitary must be considered in connexion with any test on intact immature animals. Thus the difference in effect of various gonadotrophic extracts on intact and hypophysectomized immature rats is great (see p. 77). This difference must be due to co-operation of endogenous hormones, whether or not the animal's own pituitary is abnormally stimulated by the injections. The adjustment between extract and serum may thus be complicated, and it is likely that the quantity of serum required to prevent luteinization of the membrana granulosa is increased by the necessity of neutralizing the animal's own luteinizing hormone in addition to that present in the injected extract. Experiments now being carried out in conjunction with Dr R L Noble on hypophysectomized rats are likely to show the importance, if any, of this factor

Further, it is possible, even likely, that the results cannot be explained in terms only of luteinizing and follicle-stimulating substances The possible participation of other substances in the pituitary gonadotrophic complex is suggested by the augmentation of ovarian weight response which is obtained when small amounts of the anti serum are given If this latter effect is similar to the augmentatory action previously described for anti sera to sheep and pig pituitary extracts (Rowlands 1938) it may be due to partial neutralization of the luteinizing substance of the horse pituitary extract giving rise to a ratio of L H and F S H more effective in increasing the weight of the ovary than that originally existing in the extract It might also signify the participation of the pituitary gonadotrophic antagonist postulated by Evans and others (1936), and of antibodies to it, in the results The evidence that the pituitary gonadotrophic complex may contain not merely L H and F S H but also interstitial cell-stimulating, synergistic, and antagonistic substances modifying gonadotrophic activity, each of which may perhaps be antigenic, suggests caution in offering explanations of the mechanism of qualitative action of anti-gonadotrophic sera

It is with pleasure that I acknowledge my best thanks to Dr A S Parkes F R S , for his continued interest and assistance in these studies, to Professor C R Harington, F R S , who prepared serum Prep 6, and to Dr F G Young who prepared extract AP70B used in these experiments

SUMMARY

1 Experiments on immature female rats show that it is possible to change the qualitative activity of gonadotrophic extracts of the pituitary by the simultaneous administration of anti gonadotrophic sera Two extracts of horse pituitary were used, both of which alone produced large numbers of corpora lutea in the immature ovary Two anti-sera were prepared by injecting batches of rabbits daily for periods of 3 and 5 months respectively with gonadotrophic extracts of ox pituitary

2 It was found that with suitable doses of anti serum, the luteinizing activity of the extract was completely inhibited, although the response measured by increase in weight of the ovary was only slightly altered Histologically, the response was one of pure follicle stimulation, as shown by the presence of numerous large follicles, and the absence of corpora

lutea or any luteinization of the membrana granulosa The uteri of all these animals were greatly distended with fluid, such as is found in the normal animal during oestrus Since these effects were not produced by variation in the dosage of horse pituitary extract alone, they cannot be due to a partial quantitative neutralization of the extract by the serum When larger amounts of anti serum than the optimum were given there was a progressively increasing inhibition of follicular stimulation and of the ovarian weight response

3 The significance of these results is discussed in relation to the probability that separate follicle stimulating and luteinizing hormones are present in pituitary extracts

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DESCRIPTION OF PLATES

Plate 1

FIG 1 Ovary of immature rat injected with 10 mg of horse pituitary extract (AP2B) showing corpora lutea

FIG 2 Ovary of rat injected with 10 mg of extract AP2B and with equivalent of 2 5 c c ox pituitary anti serum (Prep 6) showing follicles only Luteinization has been inhibited

FIG 3 Ovary of rat injected with 10 mg of extract AP2B and with 2 5 c c of serum A showing inhibition of luteinization

Plate 2

Fig 4 Ovary of rat injected with 2 5 mg of extract of gelding pituitary (AP 70 B), showing the presence of corpora lutea

Fig 5 Ovary of rat injected with 2 5 mg of gelding pituitary extract and 5 c c of normal rabbit serum, showing numerous corpora lutea

Fig 6 Ovary of rat injected with 2 5 mg of extract AP 70 B and 1 25 c c of serum A, showing absence of corpora lutea and of luteinization of the membrana granulosa

Plate 3

Fig 7 Uterus of rat injected with 2 5 mg of extract AP 70 B

Fig 8 Uterus of rat injected with 2 5 mg of extract AP 70 B and 1 25 c c of serum A, showing distended condition characteristic of oestrus in the normal rat

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The subthreshold potentials in a crustacean nerve fibre

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[Plates 4–6]

In a recent paper Katz (1937) has described a number of observations which are inconsistent with classical theories of excitation, but which may be explained by assuming that a subthreshold shock can elicit a small and localized action potential in the cathodic part of a nerve fibre At first the assumption of a subthreshold response seems to be in conflict with the all-or-nothing law, but there is in reality no contradiction, since the all-or-nothing principle refers to the propagated disturbance, and does not exclude the possibility of graded reactions in the stimulated region Indeed, the existence of some kind of subliminal action potential is to be expected from the local circuit theory of nervous transmission, for the current generated by the activity of a very short length of nerve would be too weak to excite surrounding regions, so that a shock which activated less than a certain length would fail to produce a self-propagating impulse and would leave behind only a small and localized response (cf Rosenberg 1937, Rushton 1937) The theory of a local response is plausible *a priori*, and it would explain a number of observations in widely different fields, but it is open to one serious objection, since many workers have searched and failed to find any electrical sign of subthreshold activity It is true that Katz (1937) showed that alternating shocks produced a residual negativity, which could be detected with a sensitive galvanometer, but it is uncertain if this was due to the summation of transient local action potentials, rather than to a persistent depolarization of the stimulated region At present, the only direct evidence for a subliminal response is provided by Auger and Fessard's (1935) studies on the excitable cells of the plant *Chara* They observed the potentials in the stimulated region and were able to record a small, wave-like response, which grew up to a propagated action potential when it reached a certain size The present paper contains an account of experiments which show that a similar result can be obtained in crustacean nerve Non-medullated nerve was used, because it was hoped that the electrical properties of the excitable membranes would be more conspicuous in the absence of a thick myelin sheath At first the whole nerve trunk or bundles of nerve fibres were employed, but these experiments failed to give any clear evidence of a subthreshold response However, during the course of the work a chance experiment showed that it was not difficult to isolate and to record action potentials from a single fibre In an isolated fibre it is comparatively easy to record from the stimulated region, and the evolution of the spike can be watched without using a balancing circuit This made the search for a local response much easier, and experiments with single fibres soon gave definite evidence of something like a subliminal action potential

METHODS

Material

Single fibres were usually obtained from the limb nerves of the shore crab, *Carcinus maenas* Most of the axons in the nerve trunk would have been too small and fragile to handle, but there are a few large fibres which can be isolated without much difficulty These consist of a transparent axis cylinder surrounded by concentric layers of connective tissue (cf Young 1936) The diameter of the axis cylinder is about 25μ and the thickness of the connective tissue about 5μ A photomicrograph of a living axon is reproduced in fig 1, Plate 4

In a few experiments single fibres were isolated from blue crabs, *Callinectes sapidus*, or lobsters, *Homarus vulgaris* Axons from these animals behave in the same way as those from *Carcinus*, but they are harder to dissect and are usually covered with several layers of diffuse connective tissue which stick to the electrodes and make it difficult to obtain a clean recording lead

Isolation of fibres

The nerve trunk was dissected from the meropodite of a walking leg and transferred to a Petri dish of sea water which was mounted on an illuminated dissecting stage Crustacean nerve is only loosely held together by connective tissue, and when immersed in sea water it floats apart into a number of thin strands, which can be separated with fine forceps or needles Most of the strands consist of small fibres and must be rejected The large fibres were identified with a microscope (magnification × 150), which was mounted on an adjustable arm, so that it could be swung over any part of the Petri dish When a large fibre had been found, the strand containing it was split into two by pulling with dissecting needles The two parts of the strand were again examined with the microscope, and the half containing the large fibre was set aside for further subdivision This process was repeated until a single fibre was obtained Sometimes the isolation of an axon could be completed in a few minutes, but often repeated subdivision was necessary The fibres were handled only by their ends, so that injury to the main body of the axon was avoided It was necessary to use a microscope for identifying the large fibres, but it was found best to view the nerve with the naked eye during the actual dissection of the fibres

Electrical recording

The essential features of the electrode system are shown in fig 2 The electrodes consisted of fine platinum hooks (A) made from 100 or 200μ diameter wire They were sealed into glass tubes (B), which in turn were mounted in adjustable bakelite bosses (C) The nerve fibre was held in position by two pairs of screw-controlled forceps (D) The electrode holders and forceps were mounted on a glass bar which could be moved horizontally about the pivot (E) or raised vertically in a Palmer adjustable stand

When the dissection was complete, the electrodes were swung over the Petri dish and lowered into the sea water One end of the fibre was placed between the tips of one pair of forceps and gripped by tightening up the screw (F) The other end was seized with a needle and the fibre drawn across the tips of the electrodes When almost taut, it was lowered into position and then gripped in the second pair of forceps The final result of this manœuvre was to stretch the fibre horizontally above the tips of the hook electrodes It was important to keep the fibre from touching the electrodes, since it might stick to them so firmly that it could not be pulled free without injury

In order to record from the fibre, it must be kept in an insulating medium At first it was raised out of the sea water into air Action potentials could be recorded in this way, but the method was unsatisfactory, since the fibre dried quickly and could not be kept out of sea water for more than a minute at a time After a few trials it was found possible to use the fibre in aerated paraffin oil Paraffin does not seem to have any harmful effects, for fibres immersed in it remain excitable and transmit impulses for several hours A layer of oil was poured on to the sea water, and the electrodes and nerve fibre were then slowly raised into it by turning the screw on the Palmer stand As the axon was brought through the interface, the surface tension caused it to stick firmly to the platinum hooks, which therefore made good electrical contact despite the surrounding paraffin

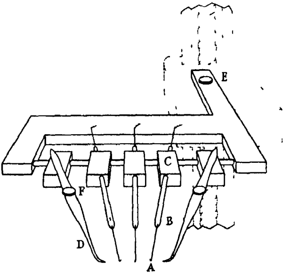

Fig 2

The principal difficulty in recording electrical changes from a single fibre is that its high resistance slows the response of the first stage of the amplifier The input resistance was kept as low as possible by working with short interelectrode distances, but it could not be reduced below about 0 5 megohm, and with an ordinary amplifier a resistance of this magnitude introduces serious distortion at comparatively low frequencies The difficulty was finally overcome by using a specially fast amplifier which was designed by Dr Toennies and which will be described by him elsewhere Tests made by calibrating through a high resistance showed that this was sufficiently rapid to follow any ordinary nerve potentials Thus when a rectangular pulse was applied through 2 megohms, the oscillograph deflexion was 90% complete in 60 μsec (fig 3, Plate 4) Calibrations made with an oscillator under similar conditions showed that the response was reduced by 7% at 5000 cycles

Grid leaks of 15 megohms were used in the first stage of the amplifier, so that no appreciable quantity of current was drawn from the nerve *

In the Cambridge experiments the fibre was stimulated with coreless induction coils, but in New York these were abandoned in favour of the thyratron discharge circuit used in Dr Gasser's laboratory This is essentially similar to the arrangement described by Schmitt and Schmitt (1932), except that the intensity of the shock is controlled by a potentiometer in the primary, instead of in the secondary circuit of the transformer Measurement showed that the shock produced in this way was 95% complete in 60μsec

During the early stages of the work various artifacts and sources of error were encountered Thus under certain conditions the nerve fibre might be stimulated by pick-up from the sweep circuit of the oscillograph, or occasionally a strong shock might stimulate through the ground lead of the amplifier Another difficulty was that the action potential might record through the capacity to ground of an idle electrode or through one of the forceps used for holding the fibre These difficulties were overcome by keeping the capacity to ground of all points connected to the nerve as low as possible Another important precaution is to maintain a very high degree of insulation between the two pairs of forceps at each end of the fibre Thus the forceps were originally attached to bakelite bosses on a metal bar, but subsequent experiments showed that a large part of the shock artifact could be traced to a leak across the bakelite insulation The metal bar was therefore replaced by one made of glass, and in this way the artifact was greatly reduced

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* Records of electrical changes in a single fibre were published in a preliminary communication (Hodgkin 1937) These were made with an amplifier which was too slow to follow rapid changes produced by a high resistance preparation, and the results, therefore, differ in a number of ways from those given in the present paper These differences are not entirely due to the relative speed of the two recording systems but are partly to be attributed to the fact that the earlier amplifier drew an appreciative amount of current from the fibre

It is important to reverse the direction of the shock by changing the polarity of the primary circuit of the transformer or induction coil If the stimulating leads are reversed, the balance of the secondary circuit with respect to ground is altered, so that the intensity of corresponding anodic and cathodic shocks may not be exactly equal The stimulating leads were connected through large condensers (1 μF) in order to avoid polarization of the nerve by potential differences developed at the electrodes

EXPERIMENTAL RESULTS

The propagated action potential in a single fibre

The propagated action potential is of constant size and form and travels without decrement at a uniform velocity of 3–5 m /sec * This result agrees with that of Bogue and Rosenberg (1936), who showed that the fastest fibres in the limb nerve of *Maia* conduct at 5 m /sec The duration of the spike usually lies between 0 8 and 1 0 msec , so that its wave-length is only about 4 mm In the *A* fibres of a frog or mammal the wave-length is about 40 mm This difference is interesting, because it may be connected with the fact that the spread of the polarization potential (electrotonus) is much less extensive in crab fibres than it is in medullated nerve The form of the spike is essentially similar to that in a medullated axon, as may be seen by comparing the record in fig 4 with the one published by Gasser and Grundfest (1936) The absolutely refractory period lasts for about 1 msec and the fibre can transmit a series of impulses at a frequency of over 500/sec Fig 5 *b* shows a train of spikes following one another at 580/sec A comparison of these action potentials with one evoked by a single shock (fig 5 *a*) shows that the potentials were reduced by about 25 % during the tetanus

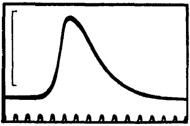

FIG 4 Propagated action potential Fibre made monophasic by crushing Time 0 1 msec Amplitude of action potential, 55 mV

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* All the experiments described in this paper were made at a temperature of 20–25° C

The most surprising fact about an isolated fibre is the size of the action potential Spikes of 40 mV were regularly observed and potentials of 50–60 mV were recorded on several occasions These large values suggest that the action potentials which are ordinarily recorded from a nerve trunk must be greatly reduced by the short-circuiting effect of the inactive tissue and interstitial fluid In a single fibre preparation much of the inactive tissue is removed, so that a higher proportion of the potential generated by the fibre is recorded But despite its large size, there is reason to believe that the recorded spike still falls far below the potential change at the nerve membrane The recorded potential would only be equal to the internal potential change if the resistance outside the nerve membrane were high compared to that of the axis cylinder, and this condition was almost certainly not realized in the present experiments For the axis cylinder was surrounded by layers of diffuse connective tissue which must have had a considerable short-circuiting effect, and this would have been increased still further by the film of sea water which adhered to the fibre after it had been raised into paraffin

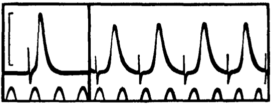

FIG 5 *a*, monophasic spike *b*, same experiment, train of spikes recorded during a tetanus Time 1 msec

The electrical changes in the stimulated region

Fig 6, Plate 5, shows the different kinds of electrical change which can be observed in the stimulated region The stimulus was a brief thyratron shock, and the potentials were recorded by connecting the distal stimulating lead to the ground lead of the amplifier

When the shock is well above threshold, it produces a large diphasic action potential which arises with no appreciable latency As the stimulus is weakened, the spike starts later and finally disappears, revealing a smaller subthreshold potential The properties of this potential are quite different from those of the electrotonic potential in a nerve trunk, and they suggest that it must be a combination of a passive polarization process and a second effect which behaves like a subliminal response of the nerve fibre

These two processes may be dissociated by varying the strength of the stimulus When the shock is weak or anodic, it affects only the polarization process, and the potential has a simple time course which is readily explained by the capacitative properties of the nerve During the period in which the shock is applied, the nerve membrane is charged very rapidly so that the potential rises abruptly, reaching a maximum in 50 μsec , after the end of the shock it subsides relatively slowly, because the charge takes an appreciable time to leak away from the membrane capacity Thus the potential has the rapid rise and gradual fall shown in G and H As long as the stimulus is well below threshold, the potential has this simple shape and the effects of anodic and cathodic shocks are symmetrical However, as soon as the shock is brought close to threshold, the cathodic potential changes in a striking and characteristic way In the first place its duration is greatly increased, and in the second it has a rounded form which is quite unlike that of the corresponding anodic polarization The simplest way of explaining these changes is to assume that the nerve produces a small and localized wave of negativity which adds to the polarization potential According to this view, we may analyse the rounded curve in E into a cathodic polarization potential which is symmetrical with the anodic one, and an additional wave of negativity which will be called a local response Fig 7 illustrates an analysis of this kind and shows that this particular local response had a rising phase of 270 μsec and a total duration of about 1500 μsec

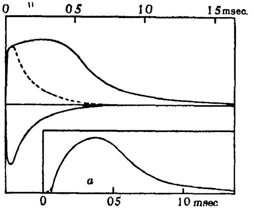

FIG 7 Cathodic and anodic potentials, traced from records in fig 6 E and F Cathodic potential analysed into polarization potential and local response

FIG 7 *a* (inset) Local response obtained by subtracting polarization from total cathodic effect

The development of the local response is illustrated by the typical experiment in fig *8a* It is clear that shocks of less than 0 5 threshold affect only the polarization process, because

(1) After 100 μsec the potential has a simple, approximately exponential decline

(2) The potential is directly proportional to the strength of the stimulus

(3) The effects of anodic and cathodic shocks are symmetrical

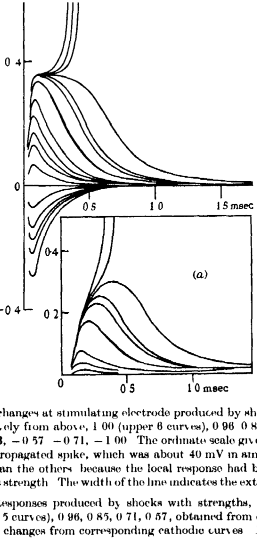

FIG 8 Electrical changes at stimulating electrode produced by shocks with relative strengths, successively from above, 1 00 (upper 6 curves), 0 96 0 85 0 71 0 57, 0 43 0 21 −0 21, −0 43, −0 57 −0 71, −1 00 The ordinate scale gives the potential as a fraction of the propagated spike, which was about 40 mV in amplitude The 0 96 curve is thicker than the others because the local response had begun to fluctuate very slightly at this strength The width of the line indicates the extent of fluctuation

FIG 8*a* (inset) Responses produced by shocks with strengths, successively from above, 1 00 (upper 5 curves), 0 96, 0 85, 0 71, 0 57, obtained from curves in fig 8 by subtracting anodic changes from corresponding cathodic curves Two of the anodic curves necessary for this analysis were recorded but are not shown in fig 8 Ordinate, as above

When the stimulus is increased beyond 0 5 threshold it produces a local response, the size and time relations of which are shown in fig 8*a* It is clear that these curves are similar to those which Katz (1937) obtained from excitability measurements In particular, both types of experiment show that the subliminal response increases in duration as well as in amplitude, and that its rising phase becomes longer as the stimulus increases

Another important property of the local response is that it starts to vary in amplitude and duration when it has almost reached propagating size Thus in the experiment of fig 8 the potentials produced by successive

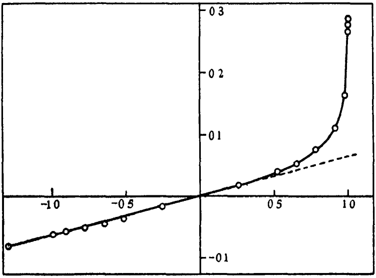

Fig 9 Relation between potential at 0 29 msec after application of shock, and strength of shock Ordinate, potential as fraction of spike potential Abscissa, strength of shock as fraction of threshold

shocks were constant to within 1 % provided that the stimulus was below 0 96 threshold However, when it was increased beyond this point, the responses started to fluctuate over a wide range and ultimately a few succeeded in growing up into propagated spikes The transition from local to propagated responses seems to occur at a constant level, for if the responses succeed in reaching a critical size, they turn into propagated spikes, whereas when they fail to attain it they die out as localized monophasic waves (cf especially fig 10) Although it is impossible to give a precise explanation of the fluctuations in the subliminal response, it is not difficult to understand their general nature For it has been known since the work of Blair and Erlanger (1933) that the threshold varies continually

FIG. 1. Living axon from *Carcinus* in sea water. The light intensity was adjusted so as to produce the maximum contrast between the opaque connective tissue and the transparent axis cylinder.

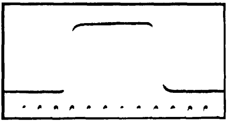

FIG. 3. 10 mV applied to amplifier through 2 megohms. Time 0·1 msec.

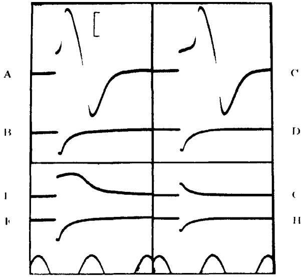

Fig 6 Electrical changes at stimulating electrode produced by shocks with relative strengths A 1·05 B −1·05 C 1·00 D −1·00 E 1·00 F −1·00 G 0·61 H −0·61 Time 1 msec The difference between C and E was due to a slight fluctuation in excitability since the shock strength was not changed between making these two records Scale 15 mV

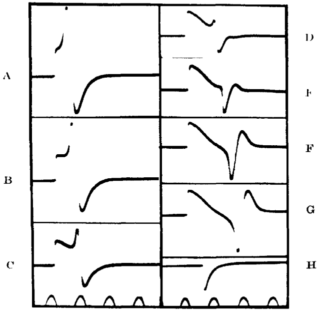

Fig 19 Spikes recorded diphasically with ground lead connected to cathode and grid lead 1·0 mm distal A shock = 1·08 threshold B 1·03, C–G 1·00, H, anodic polarization produced by shock with strength −1·00 Time, 1 msec

in a nerve fibre, and it is clear that a slight fluctuation in excitability might cause large changes in the local response, since this increases very rapidly near threshold Blair and Erlanger (1936) found that the threshold varied spontaneously over a range of 10 % In an isolated crustacean axon the threshold does not usually fluctuate over more than ½ %, but some play can always be detected Now this slight shift in excitability would not produce appreciable variations when the stimulus is small, but it should cause large fluctuations near threshold, since a slight change in stimulus then produces a relatively enormous change in the local response

The rapid increase in the local response as the shock approaches threshold is illustrated in fig 9 It is clear that anodic or weak cathodic shocks produce only polarization, since the potential is at first directly proportional to the strength of the stimulus The response seems to start at about 0 5 threshold, but it is rather hard to tell exactly where it begins since the initial curvature in fig 9 is very gradual At 0 7 threshold the response is quite definite, and after this it increases at accelerating rate until it finally reaches propagating size

Local response and latency

One of the most important properties of the local response is that its time relations determine the latency of the nerve fibre The reason for this is that the spike initially follows the same time course as the local response, so that the latency of a response which just succeeds in propagating is approximately equal to the time of rise of one which just fails This point is illustrated by fig 8, but it is more clearly shown when the latency is long, as in fig 10 Here it is evident that both local and propagated responses start out in the same way and may not separate until nearly 500 μsec after the shock The final division of propagated and local responses occurs at a critical potential, for all the responses which reach this potential turn into propagated spikes, whereas those which fail to attain it die out as localized monophasic waves

The association between the latency and the form of the local response is very constant, and has been checked in a large number of experiments Usually the spike does not start later than the crest of the response, but occasionally it may rise when the wave has fallen a little below its maximum. An example is shown in fig 11 Here the latency is increased to over 1 msec and the local response has a correspondingly long duration In this case the response does not follow a smooth curve, but is prolonged by the development of a hump on the falling phase This secondary wave seems to be associated with the long latency, since the delayed spikes start near its crest At first it is rather difficult to see why the spike should start after the peak of the local response, and this problem becomes more acute when we remember that the response is superimposed upon the decaying cathodic polarization, so that the spike actually starts when the potential has fallen far below its maximum (fig 11*a*) One possible explanation is that both the secondary humps and the spikes which rise from their crest are due to the development of activity at a short distance from the cathode

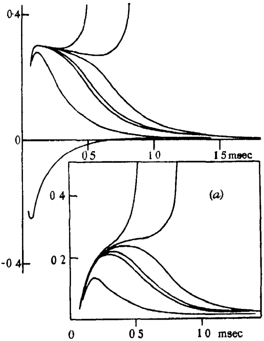

Fig 10 Electrical changes produced by shocks with relative strengths 1 00 (upper 5 curves), 0 97, −0 97, −1 00 (these two anodic curves coincide)

Fig 10*a* (inset) Responses evoked by cathodic shocks in fig 10 The ordinate scale in both figures gives the potential as a fraction of the spike

This would account for the fact that the spike begins when the potential at the cathode is falling, since it does not actually start from there, but at a distant point where the potential is still rising, or just reaching its crest The evidence for this hypothesis will be presented later, but it is well to point out now that the short latency type of response is probably the more normal one Thus the long latencies are usually found in fibres which have been badly dissected or which have been stimulated for some time Figs 8 and 10 illustrate the effect of stimulation on the latency, since both were made on the same axon, but at different times, fig 8 being made at the beginning of the experiment, and fig 10 after 20 min of stimulation

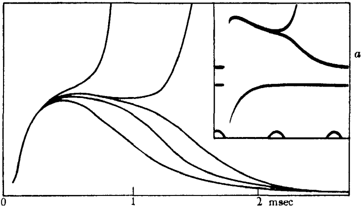

FIG 11 Local and propagated responses in a nerve with a very long latency, obtained in the usual way by subtracting polarization from total effect produced by cathodic shock All curves made with same strength of stimulus

FIG 11*a* (inset) Three of the original records used for making analysed curves in fig 11 Upper two curves effects produced by threshold cathodic shocks Lower curve anodic polarization produced by reversing shock Time 1 msec The hump on the local response seems to be larger in the original records than in the analysed curves This is an illusion depending on the fact that the inflexion on the local response is exaggerated by being superimposed on the concave polarization potential

Possible latency of local response

The experiments described in the previous section do not give a complete picture of nerve latency, but they suggest that it is connected with the time required for the local response to grow into a self propagating impulse, rather than with delay in the activation process itself Thus it is clear from fig 10*a* that only a very small part of the spike latency could possibly be due to a delay in the initiation of the local response most of it is due to the time taken by the response to reach its maximum

The shape of the local response at first suggests that it may have a very small but measurable latency since it starts with a delay or inflexion lasting for 50-80 μsec It is possible that some of this inflexion is due to a latency or inertia in the activation process, but a large part of it must arise in other ways In the first place, we should expect the local response to have a gradual rather than an abrupt beginning, because the shock does not charge the nerve instantaneously but over a period of about 60 μsec Secondly, even if the response had an abrupt start, we should not be able to measure it, because the lag in the amplifier would delay the rise of the potential during the first 50μsec Thirdly, it is important to remember that potentials are recorded from the distal side of the cathode, whereas the stimulus first acts on the proximal side Now owing to the time required to charge the membrane capacity, any potential must take a certain time to spread across the nerve under the electrode, so that the recorded potential would lag behind the one at the cathodic point This consideration also explains why the recorded polarization potential sometimes reaches its maximum after the end of the applied shock

The local response in the refractory period and supernormal phase

The distinction between the subliminal response and the polarization potential can be brought out most convincingly by studying their behaviour in the refractory period It is found that the ability to produce a subliminal response is greatly reduced during the refractory period, so that a shock which normally evokes a large local response may produce only polarization if applied to refractory nerve This is illustrated by fig 12 Record A shows the electrical change produced by a just subthreshold shock, and B the corresponding anodic polarization C and D were made a few seconds later and show the effect of applying the same shocks in the relatively refractory period It is clear that the local response is practically abolished by making the nerve refractory, for the cathodic potential no longer has a rounded form and is only slightly larger than the corresponding anodic potential On the other hand, the polarizable properties of the nerve do

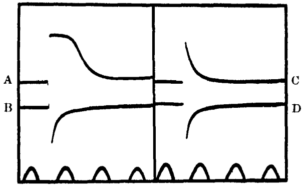

FIG 12 A Effect produced by just subthreshold shock in a resting nerve B Anodic polarization produced by reversing shock in A C Same shock as in A applied to refractory nerve D Same shock as in B applied to refractory nerve Time 1 msec The nerve was made refractory by a shock which preceded the testing stimulus by 2 5 msec At the moment when the second shock was applied the threshold was 119% normal Potentials recorded at stimulating electrode

not seem to be much changed, since the anodic potentials in B and D are almost identical Actually the potential in D is 2 or 3 % less than that in B, and it has usually been found that the polarizability is slightly reduced for several milliseconds after the spike

In this experiment the fibre was left for several hours in sea water before being used This was done in order to prolong the relatively refractory period, so that records C and D should not be complicated by the conducted phase of the conditioning spike

The excitability following an action potential usually goes through a pronounced supernormal phase, so that it is easy to observe the effect of supernormality on the local response As we should expect, the ability to respond is greater during the supernormal phase, and a large local response can be produced by shocks which are too weak to elicit any response in

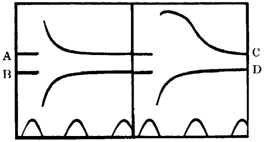

FIG 13 Potentials produced by A Shock with strength 0 76 resting threshold applied in resting nerve B Anodic polarization produced by reversing shock in A C Same shock as in A applied in supernormal phase D Same shock as in B applied in supernormal phase Time 1 msec Nerve made supernormal by shock applied 6 7 msec before second stimulus, the threshold was 76 % normal at moment when second shock was applied Potentials recorded at stimulating electrode

normal nerve Fig 13 illustrates this point In this experiment there was practically no difference between the anodic potentials in normal and in supernormal nerve, but in others the polarizability was sometimes slightly reduced during supernormality

The evolution of the local response in the refractory period is essentially similar to that in normal nerve, although of course it does not develop until the shock is increased beyond the normal threshold This is illustrated by fig 14, which shows that the potential increases rapidly before the refractory threshold, in the same way as it does before the normal one This experiment brings out another interesting point, since it shows that the potential at which the local response turns into a propagated action potential is increased during the refractory period This seems reasonable, since it must be harder for the action potential to excite refractory nerve, and therefore the local action potential would have to be larger in order to propagate away from the cathodic region

The position in the supernormal phase is the exact opposite of that in the refractory period, since it is then relatively easy to set up a local response and this propagates at a potential which is lower than that in a normal nerve This is illustrated by fig 15

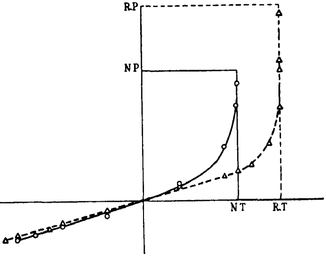

FIG 14 Broken curve triangles Subthreshold potentials in refractory nerve Continuous curves, circles Subthreshold potentials in resting nerve Abscissa strength of shock Ordinate potential, measured at 0 5 msec after the shock Vertical lines $N\ T$ and $R\ T$ give the normal and refractory thresholds respectively The horizontal lines $N\ P$ and $R\ P$ show, approximately, the potentials at which the local response starts to propagate in normal and refractory nerve

The relation between the potential at which the local response propagates and the excitability is illustrated by an experiment in which threshold responses were measured successively in supernormal, resting, and refractory nerve (fig 16) In the first two cases the form of the response was obtained in the ordinary way, by subtracting the polarization from the total effect produced by the cathodic shock The procedure for measuring the refractory response was slightly more complicated, because the potentials were superimposed upon the curved base line left by the conditioning spike It was therefore necessary to combine three records in the following way

$$(A-B)+(C-B),$$

where A = first spike + cathodic polarization + response, B = first spike alone, C = first spike + anodic polarization The curves in fig 16 show

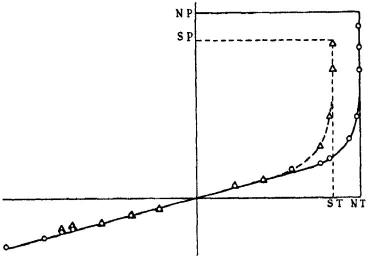

FIG. 15. Broken curve, triangles: subthreshold potentials in supernormal period. Continuous curve, circles: potentials in resting nerve. Abscissa: strength of shock. Ordinate: potential measured at 0·47 msec. after shock. *N T* and *S T* are normal and supernormal thresholds. *N P* and *S P* indicate potentials at which response propagates in normal and in supernormal nerve.

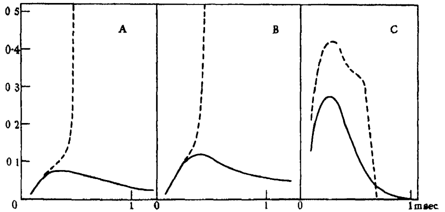

FIG. 16. Local and propagated responses recorded at cathode, under different experimental conditions. Response measured by subtracting anodic polarization from total effect produced by cathodic shock. Dotted lines: spikes which just succeed in propagating. Continuous curves: largest local responses observed. A. Supernormal nerve, 1·7 msec. after beginning of conditioning spike. Strength of shock = 82 % resting threshold. B. Resting nerve. C. Relatively refractory nerve, 0·9 msec. after beginning of conditioning spike. Strength of shock = 218 % resting threshold. The ordinate gives the potential as a fraction of the spike in a resting nerve. The spike amplitude was about 40 mV.

clearly that the potential at which the response propagates increases as the threshold rises so that a refractory response may fail to propagate, although twice the size of the largest local response in a resting nerve It is interesting to note that the spike is about eight times larger than the subliminal response in a resting nerve, whereas in a refractory nerve it may be only 1 5 times as great This result suggests (1) that the spike normally has a large reserve of electrical energy at its disposal, and (2) that this reserve is greatly reduced during refractoriness

Fig 16 shows that the refractory spike does not follow a smooth curve, but has a hump on the descending phase Discontinuities of this kind are frequently seen in refractory nerve, and they may occur on the local response as well as on the spike It is probable that they are due to the development of activity at a short distance from the cathode (see p 114 *et seq*)

Relative amplitudes of spike and threshold local response

The difference between the propagated and the local action potentials can be reduced in other ways besides making the nerve refractory Thus it decreases if the fibres are placed in K rich solutions or left to soak in sea water, or in fact if they are maltreated in any way Under these conditions conduction ultimately fails, and it is then often difficult to decide whether a response is to be regarded as a spike which has been blocked, or a large local response which is unable to propagate away from the cathodic region

Another important factor is the interelectrode distance for the threshold local response increases as the stimulating electrodes are brought closer together This result seems reasonable since the polarization disappears more rapidly with a short interpolar distance, so that it can give less support to the developing response, which must therefore be larger in order to propagate throughout the fibre

Owing to the variations introduced by these factors, it is impossible to give any precise figure for the ratio of the spike to the threshold local response, but it is usually of the order of 5, as may be seen from the series of experiments in Table I

The third column in this table gives the amplitude of the polarization produced by a threshold shock This cannot be measured directly, since the peak of the cathodic polarization is complicated by the beginning of the local response It may, however, be estimated quite accurately, by reversing the direction of the shock, and measuring the amplitude of the pure anodic polarization This procedure is justifiable, since the cathodic and anodic polarizations produced by weak shocks are always mirror images of one another

Another quantity which is of some interest is the potential at which the local response first becomes appreciable. This can be measured directly by recording the electrical changes produced by increasing strengths of shock. Thus in the experiment of fig. 8 the local response started when the shocks were increased to 0·57 threshold, and at this strength they produced a cathodic polarization which was equal to 0·17 of the spike height. Direct measurements of this kind are given in some of the experiments in column 4; in the others a complete series of subthreshold curves was not made, so that the potential could not be measured directly. However, a rough estimate could be formed from the figures in column 3, for the local response always begins somewhere between 0·5 and 0·8 threshold, so that it must start at a polarization which lies between 50 and 80% of the threshold one.

TABLE I

Data obtained from experiments made during December 1937 and January 1938. All experiments in which the necessary measurements were made have been included. 7 *a* and *b* were obtained from the same axon at different times, otherwise measurements made on separate fibres. Column 3 obtained by reversing threshold shock and measuring anodic polarization. Column 4: ratio of polarization at which local response starts, to spike potential; unbracketed figures measured directly, bracketed figures, limits calculated as 0·5–0·8 threshold polarization.

	1 Spike amplitude mV	2 Local response Spike	3 Threshold polarization Spike	4
1	39	0·21	0·21	(0·10–0·16)
2	36	0·17	0·21	0·11
3	52	0·15	0·28	0·14
4	34	0·20	0·23	0·14
5	52	0·19	0·20	(0·10–0·16)
6	44	0·18	0·20	(0·10–0·16)
7*a*	—	0·30	0·29	0·17
7*b*	—	0·24	0·25	0·17
8	43	0·29	0·31	(0·15–0·24)
9	40	0·12	0·16	(0·08–0·13)
10	56	0·18	0·20	(0·10–0·16)

The results in column 4 are interesting in connexion with the rising phase of the spike. If transmission is electrical, the initial part of the spike should be due to current spreading ahead of the active region, and the activity under the lead should not start until the nerve is depolarized to a critical extent. Column 4 indicates that, with an external stimulus, the activity does not start until the nerve is depolarized to about one-seventh spike height. If this result is applied to a propagated impulse, it follows that the active generation of potential should start when the action potential rises to one seventh of its maximum, and that below this the rise in potential should be solely due to the physical spread of polarization ahead of the active region

The estimate of one seventh is likely to be too large, because controls with dead fibres show that about 20 % of the polarization which is recorded arises in the stimulating electrode and not in the nerve fibre itself However, it seemed best to give an estimate which was an average of experimental values, rather than to add an uncertain correction for electrode polarization

Possible sources of artifact—Electrode polarization

The fact that the local response can be abolished by making the nerve refractory proves that it is a product of the nerve but this does not exclude the possibility of the polarization potential being complicated by artifacts It is, therefore, important to consider the various types of artifact which could be observed In the first place, there might be a capacitative disturbance due to coupling between the leads This effect was nearly always present and was rather serious in the earlier experiments, but it could be distinguished from the polarization potential, since it consisted of a quick spike lasting for less than the shock duration As the work progressed this was gradually reduced and it might be completely absent when the interelectrode resistances were low Thus the records in fig 6 Plate 5 were made with a particularly large fibre, and as a result they are free from any initial artifact In the other experiments illustrated there was an initial disturbance in the original records, but this was too faint for reproduction, so that there is a gap of 30 or 40 μsec in the oscillograph line

The second possibility which must be considered is that the potentials might be complicated by polarization in the common stimulating and recording electrodes In order to determine the importance of electrode polarization, various control experiments were made on dead nerves It was found that if the fibres were left for 7 or 8 hr in sea water, they became completely inexcitable and failed to give any trace of a local or propagated response Under these conditions the shocks produced a perfectly symmetrical polarization potential which might not be much smaller than that in a normal nerve This potential gradually declined as the fibre was left in sea water, and after prolonged soaking or after treatment with dilute alcohol it was reduced to 15–25 % of its original value, so that at least 75 % of the polarization observed must have been in the fibre and not in the electrodes The remaining 25 % was probably due to electrode polarization, but it is difficult to be sure that there is not some residual polarization in a dead fibre These experiments show that most of the polarization is in the nerve, but they do not prove that the accumulation of charge occurs at the membrane involved in excitation Information on this point was obtained by comparing the polarizability during the resting and active states It was found that a shock applied at the crest of the spike produced less than half the normal polarization This proves that a large part of the polarizability of the fibre must be due to the excitable membrane, since it would not otherwise be affected during activity The experiment also has a more general interest, as it affords new evidence for the theory that the action potential arises by the breakdown of a polarized membrane

The spatial distribution of the subliminal response

The principal difficulty in measuring the distribution of the subliminal potentials in a single fibre arose from the fact that they occupy an extremely short length of nerve It was therefore impossible to use a series of fixed electrodes, and it was necessary to record from a single electrode which could be moved along the nerve fibre A satisfactory movable electrode was made by attaching one of the electrode holders (*B*) (fig 2) to a strip of phosphor bronze, which in turn was connected to the bakelite boss (*C*) The motion of the electrode could then be smoothly controlled by means of a screw which was arranged to press against the phosphor bronze strip This arrangement worked fairly well, provided that the fibre could be dissected free from all loose connective tissue, but if this was present the electrode stuck to the fibre and could not be moved without injuring it The distance between the stimulating and recording electrodes was measured by observing them with a microscope into which a micrometer scale had been inserted This method was an accurate one but the measurement of distances less than a millimetre is uncertain since the electrodes were 100μ in diameter and were, moreover, effectively broadened by the film of sea water which collected at their point of contact with the nerve The distance between the electrodes was measured between their distal edges, since the potentials probably record from these points

At first the distribution of potentials was measured with an ordinary grounded amplifier, but this method proved to be unsatisfactory, on account of the inevitable capacity between the stimulating circuit and the ground lead This capacity led to two undesirable results In the first place it had the effect of making the strength of the shock dependent upon the position of the ground lead, so that the stimulus was altered by a small but unknown amount as the ground lead was moved along the nerve This effect depends stated in vague terms Thus it may be that the spread of the local response is due to an increase in length of the active region, as well as to the passive current spread which operates in the case of the anodic polarization The extension of the active region must in the first instance depend upon a spread of polarization, but it would clearly enable the response to spread much further than a polarization process alone If this view is correct, it follows that the spread of the local response must be fundamentally unlike that of the polarization, and is more closely allied to the propagation of the spike

It is difficult to distinguish experimentally between the active and passive types of spread, but there is evidence to show that the active region may sometimes extend over appreciable lengths of nerve Thus it has been found that under certain conditions the subliminal response may spread for relatively enormous distances along the fibre, whereas the spread of the anodic polarization remains perfectly constant An example of this is shown in fig 18 Here the response at 1 5 mm is nearly as large as it was at the cathode, whereas the anodic polarization is so small that it can hardly be measured The prolonged duration and peculiar shape of the response in A are characteristic of fibres in which the subliminal wave spreads for a long way This experiment is satisfactory in so far as it illustrates the difference between the polarization and the subliminal response, but it immediately raises the question as to why the response should sometimes spread to such unusual extents One possible explanation is that there may occasionally be a gradient of excitability along the fibre In this case, the normal tendency to decrement would be counterbalanced by the fact that the response was continually working itself into more excitable nerve and so could more easily induce a response in the next section

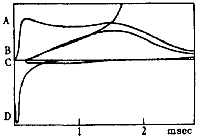

FIG 18 A Just subthreshold response at stimulating electrode B Just subthreshold and just superthreshold responses at 1 5 mm distal Strength of shock = 98 % of that in A C, D Anodic polarizations at stimulating electrode and at 1 5 mm produced by reversing shocks in B and A

Spread of local response and long latency

The widely spreading subliminal responses described in the previous section are interesting because they only occur in fibres which have a long latency Fig 18 B illustrates this association, for the maximum delay of the spike was 1 3 msec, and of this only 0 3 msec could have been due to conduction time because the recording lead was 1 5 mm from the cathode At first the connexion between an increase in latency and an increase in spatial spread seems obscure, but the two curves in fig 18 B suggest a plausible explanation for the association Thus it is possible that the subliminal response might travel along the nerve and finally work up into a spike in the more excitable nerve at a distance from the cathode This hypothesis would account for the fact that the spike arises near the crest of the subliminal response in B More direct evidence may be obtained by recording with both leads close to the cathode since it is then possible to determine the place from which the spike starts The records in fig 19, Plate 5, show the results of an experiment in which the ground lead was connected to the cathode and the grid was placed 1 0 mm away Now if the spike arises at, say, 0 7 mm from the cathode, it must reach the grid lead before it has time to propagate back to the cathode, so that instead of obtaining a normal diphasic impulse, we shall record one in which the two phases are reversed This effect can be seen in F and G, but it occurs only when the latency is exceptionally long, as this decreases, the response starts progressively closer to the cathode Theoretically we should expect to record only a minute change when the response starts midway between the leads, but this result is never obtained in practice, since the spike is always slightly larger at one lead or the other, so that a potential like D is recorded The wide shift in the point of origin of the spike is at first a little surprising, since we might expect that the spike would always start from the distant points if these were more excitable than the rest of the nerve The reason for the shift is that the crest of the subliminal response travels very much slower than the spike, so that if the shock is strong enough to excite at the cathode, the spike starts from there with a short latency and travels along the nerve before the subliminal response has had time to spread for any distance

Records like those in fig 19 could be observed in most experiments in which the latency was longer than 500 μsec, but exceptions have occasionally been found These can be explained quite simply by supposing that the spike is still starting at a distance from the cathode, but in the interpolar, instead of in the extrapolar region If this happened, it would clearly be impossible to observe any "reversed" action potentials, since the activity must always reach the ground electrode before it arrives at the grid If this explanation is correct, it should become possible to record a reverse action potential effect on interchanging the grid and anode leads (fig 20) This deduction has been verified in the few cases in which it has been possible to test it In most cases, however, there has been no need to make the test, since the delayed spikes usually start in the extrapolar stretch of nerve

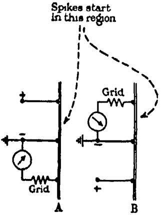

FIG 20 A Spike starting in interpolar region, no reversal of phases possible B Grid and anode leads interchanged, now possible to record reverse spikes

The experimental analysis of the long latencies may be summarized by saying that the following changes occur in association

(1) The local response spreads for a long way

(2) It is unusually prolonged and often has a hump on the descending phase

(3) Delayed spikes start at a distance from the cathode

(4) The latency is long

It is easy to account for all these changes, if we assume that they occur only when the excitability beneath the cathode is slightly lower than in a neighbouring part of the nerve In this case, the local response would spread for a long way, because it would be working into more excitable regions, it would last for a long time, because the negative wave at one point would be maintained by the development of activity in a more distal part, the spike would tend to start at a distant point, because the excitability there would be greater than underneath the cathode, and finally there would be an increased latency, because the local response at a distance takes a long time to work up to propagating size

This explanation receives some additional support from the nature of the conditions that promote long latencies Thus they tend to occur after prolonged stimulation or in fibres which have been stretched too tightly across the electrodes Another way of obtaining long latencies is to place the leads near the killed end of the fibre Now we should expect that all these conditions would tend to give an irregular distribution of excitability In the first case the cathode might become depressed as a result of some locally injurious effect of the stimulus and in the second, as a result of direct mechanical contact with the electrode In the third case we should expect a continuous gradient of excitability rather than a local depression at the cathode, but it is clear that this should be equally effective in promoting a long latency

The fact that spikes which start with a long latency arise at a distance from the cathode, raises the question of whether there may not always be a similar but smaller shift in the point of origin of the spike It is true that in a normal nerve there should be no gradient of excitability to favour points at a distance from the cathode but we should nevertheless expect some effect of this kind For the local response must be due to the activity of a certain length of nerve, and the spike should arise at the edge of this region rather than from the centre, which may indeed be completely active already In order to obtain information on this point, a few experiments were made with the recording leads very close together, but the results soon showed that it was impossible to measure the exact point of origin of the spike unless the latency was exceptionally long Owing to surface tension effects it was difficult to bring the grid lead closer to the cathode than 0 3 mm , and at this distance ' reversed ' action potentials were not obtained until the latency exceeded 500–800 μsec These spikes must rise at about 0 15 mm from the cathode, so that action potentials starting with a normal latency of 200 μsec would almost certainly rise within less than 0 1 mm of the cathode– a distance too small to introduce any detectable interaction or reversal of the two phases of a diphasic spike The question of the exact point of origin of the short latency spikes must, therefore, be left open for the moment

Further properties of refractory local responses

In an earlier section, it was shown that it was more difficult to elicit a subliminal response in the relatively refractory period, and that this had to be larger before it could propagate Another important property of the refractory local response is that it may spread for relatively large distances This effect was first discovered in an experiment in which the refractory local response was occasionally conducted as far as the grid electrode, thus producing a diphasic subthreshold wave Similar results obtained in a later experiment are shown in fig 21 These records illustrate certain other properties of the refractory response which must now be discussed In the first place it is clear that the variation in the size and form of the local response is much greater in the refractory period than in resting nerve This is to be expected, for the refractory responses must be influenced by random variations in the rate of recovery as well as by the play of excitability The second point is that the local response may either be perfectly smooth, as in C and D, or it may have a secondary wave rising from its crest, as in E–I These extra waves find a parallel in the humps which are occasionally recorded in resting nerve (cf fig 11) and it now seems that they may be due to the development of activity at a short distance from the cathode In a normal resting nerve the response decrements rapidly, but in the refractory period this tendency is counteracted by the fact that the response is continually working itself into more excitable nerve If recovery were perfectly uniform throughout the fibre, we should not anticipate such definite humps as those in fig 21, but we should expect to find them if the local response suddenly encountered a patch of nerve where the recovery was much more advanced than elsewhere In this case the activity would immediately start to spread faster and the potential would rise steeply in the whole of the cathodic region If the rise in potential were large enough, the response would be able to sustain itself by restimulation and would ultimately propagate throughout the fibre (I) On the other hand, if it were not sufficiently strong to overcome the resistance offered by the more refractory nerve on the other side of the excitable patch, the wave would die out and would produce a monophasic hump like that in F The experimental evidence for supposing that the secondary humps are due to activity spreading away from the cathode is as follows The positive wave in G can only be explained by supposing that activity has spread over to the grid electrode The negative humps occurring in E and F are plainly related to the positive waves in G and H Thus both have the same general shape and the production of a positive wave depends upon the occurrence of a large negative hump It therefore seems reasonable to suppose that both the negative and the positive humps are due to spread of activity and that the only difference between them is that the activity occurs closer to the cathode in the first case, and to the grid lead in the second

This experiment shows very clearly that there is no fundamental difference between a wave of activity which just succeeds in propagating and one which just fails Thus the response in I at first follows a course which is similar to that in H, and it is only when activity has spread to the grid electrode that the greater initial size of I finally enables it to grow up into a propagated spike Indeed it is impossible to be absolutely certain that H failed to propagate, since it is conceivable that it might have developed into a spike at some point beyond the grid electrode

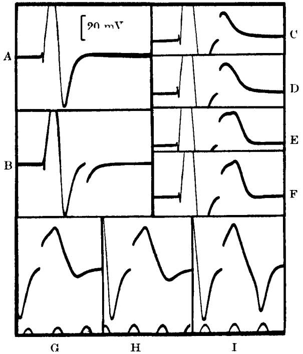

Fig 21 A Conditioning spike alone B Conditioning spike + anodic polarization produced by shock with strength − 1 5 resting threshold C–I Conditioning spike + effect produced by shock with strength 1 5 resting threshold Varying amounts of response Time, 1 msec The conditioning spike was 50 mV in amplitude The grid lead was 1 5 mm distal to the ground lead The conditioning spike has been retouched

The similarity between subthreshold and superthreshold activity becomes most striking when we study the action potentials set up early in the relatively refractory period In a resting nerve the propagated action potential is about five times as large as the subthreshold one, but in the refractory period the normal safety margin may be reduced to a point where a superthreshold wave is imperceptibly larger than a subthreshold one An example of this is shown in fig 22 *a* These curves were made with a shock of constant strength, but owing to slight changes in the rate of recovery, the size of the response varied from shock to shock The superthreshold waves are sharply distinguished by the presence of a conducted phase, but apart from this it is impossible to see any fundamental difference between propagated and local responses Indeed, in the limiting cases of the waves which just succeed and which just fail to propagate, the two curves do not begin to diverge until after the maximum of the cathodic response This result suggests that both sub- and superthreshold waves at first spread along the nerve in the same manner, and that they do not finally separate until activity has travelled for a certain distance from the cathode In the experiment illustrated it was possible to verify this conclusion by recording the activity at different points on the nerve, and some of the results are reproduced in the other sections of fig 22 Tracings of the actual records are given in the left-hand column, while the curves in the right-hand column show the refractory responses uncomplicated by polarization or conditioning impulse A large number of records were made at each point, but except in A and C only the critical cases of the waves which just succeed and which just fail to propagate are given

It is interesting to note that the anodic polarization decrements rapidly along the nerve whereas the amplitude of the response declines very little during the first 0 7 mm As indicated previously this capacity for spreading without decrement depends upon the balance of the following two factors

(1) The response tends to decrement because it is moving away from nerve which has been polarized cathodally by the shock

(2) It tends to increment because it is moving into nerve where the recovery of excitability is more advanced

The ultimate fate of the response depends upon the conflict between these two factors If the first one dominates, the activity dies out as a monophasic wave, whereas if the second gains the upper hand the activity flares up into a propagated disturbance

It was not always possible to obtain complete convergence of the subthreshold and superthreshold responses during the refractory period Often the superthreshold wave had a hump which was not shown by the subthreshold one, so that the situation at the cathode resembled that at 0 4 mm in fig 22 *c* This type of response seemed to occur more frequently when the recovery of excitability was very rapid, and we should expect to find it under these conditions, since the sub- and superthreshold waves would then separate much sooner and much closer to the cathode than they do in fig 22 When this happens there can be no possibility of any convergence, since the superthreshold wave at the cathode must always be

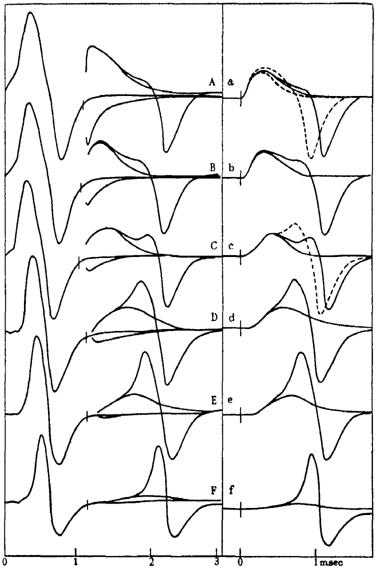

FIG 22 Refractory responses and polarization recorded at different distance from cathode A–E Tracings of records showing upper line, conditioning spike + cathodic polarization + response middle line, conditioning spike alone, lower line, conditioning spike + anodic polarization *a–e* Corresponding responses obtained by subtraction A, *a*, at cathode, B *b* 0 3 mm distal C *c* 0 4 mm distal D, *d*, 0 7 mm distal, E *e*, 1 0 mm distal, F, *f* 1 4 mm distal The strength of the second shock was 3 8 × threshold Except in *a* and *c* only the critical cases of the waves which just succeed and which just fail to propagate are given The additional responses in A and C are marked by dotted lines

increased beyond the subthreshold one by the development of the spike at a short distance away

In the experiments of figs 21 and 22 the refractory and conditioning action potentials were started by shocks applied to the same pair of electrodes Similar results have been obtained in other experiments, where the first impulse was started from a separate pair of electrodes, so that there is no reason to believe that the results were complicated by any after-effects left by the first shock

Subthreshold activity during a tetanus

The subthreshold potentials during a high frequency tetanus are similar to those in the refractory period of single spikes Fig 23, Plate 6, shows a series of potentials recorded from the stimulating electrode during the application of a train of thyratron shocks In A the shocks were cathodic, so that the records consist of propagated and local action potentials superimposed upon the passive polarization produced by the shocks The direction of the shocks was reversed in B and the potentials then consist only of anodic polarization and all or-nothing spikes which have travelled along the fibre from the other stimulating electrode This experiment is interesting for two reasons In the first place it illustrates the great variety in the size and form of the refractory potentials Thus in some cases the subliminal responses are smooth and continuous, while at other times they have secondary waves rising out of them These additional waves are extremely variable, for they may be large and diphasic as in *a* or small and monophasic as in *b*, or again they may arise late, on the descending phase of the potential, as in *d*

In the second place the experiment is important because it affords clear evidence of a refractory period following the local response Thus when the response is small, as in *c*, the succeeding spike is large and arises with no delay On the other hand, when the subliminal response is large, as in *b*, the next spike is subnormal in amplitude and starts after an appreciable latency Finally, when the subliminal wave is large enough to spread to the grid electrode (*a*), the succeeding shock fails to excite and produces only a small local response

The results obtained with high frequency stimulation recall an observation made by Gasser and Grundfest (1936) They studied the effect of tetanizing mammalian nerve and showed that it could follow 2000 shocks per sec in the cathodic region, whereas more distant parts failed to respond to these high frequencies It now seems possible that their cathodic

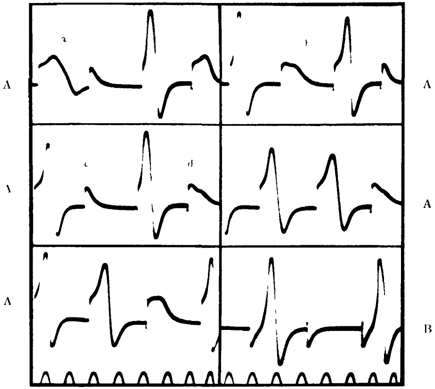

FIG 23 Potentials during a tetanus recorded with ground lead on stimulating electrode grid lead approximately 1·5 mm distal The records were photographed at intervals of 2 or 3 sec A cathodic shocks B, anodic shocks Time 1 msec

response must have been due to local action potentials of the same type as those observed in the present research

DISCUSSION

Katz's (1937) experiments suggest that a local response plays an important part in the excitation of medullated nerve, so that the failure of previous workers to record any kind of local action potential is at first rather surprising There are, however, a number of reasons for believing that it would be very difficult to observe subthreshold responses in any multifibre preparation In the first place, the maximal amplitude of the subliminal response in a crustacean fibre may be only one-tenth of that of the spike, and its size varies inversely as the excitability, so that the subliminal responses in the axons which respond at threshold in a nerve trunk would be exceptionally small Secondly, since the local response develops very rapidly near threshold, it follows that a just subthreshold shock would produce appreciable local action potentials in comparatively few fibres in the nerve trunk Thus the total subthreshold response would be extremely small compared to a maximal spike Moreover, the response would not possess the definite characteristics which it has in a single fibre, since it would be composed of a number of local action potentials, whose shape and size must vary with the excitability of each axon Finally it would be difficult to observe this small and ill-defined response, because it would be superimposed upon the large polarization potential arising from all the fibres in the nerve trunk

It is a pleasure to express my deep gratitude to Dr Gasser and the members of his laboratory for assistance and encouragement during the course of this work I also wish to thank Dr Pantin for demonstrating the dissection of the limb nerve in *Carcinus*

SUMMARY

Experiments with isolated crustacean nerve fibres show that subthreshold shocks produce two quite distinct effects When the stimulus is weak and cathodic, or anodic and of any strength, it produces a "polarization" potential which behaves as though it were due to the passive accumulation of charge at the nerve membrane On the other hand, when the stimulus approaches threshold, it produces an additional wave of negativity which

behaves like a subliminal response of the nerve fibre These two processes have entirely different properties Thus

(1) The size of the polarization potential is directly proportional to the strength of the shock, whereas the subliminal response increases at an accelerating rate as the shock approaches threshold

(2) The time relations of the processes are different The polarization potential rises during the period in which the shock is applied and then declines in an approximately exponential way On the other hand, the subliminal response starts with an initial inflexion and continues to grow for as much as 500 μsec after the end of the shock

(3) The time relations of the polarization potential are independent of the strength of the shock, whereas the duration of the local response increases and its maximum occurs later as the shock approaches threshold

(4) When the local response has almost reached propagating size, it becomes unstable and varies from shock to shock On the other hand, the polarization produced by successive shocks is always quite constant

(5) The local response can be greatly reduced or abolished by making the nerve refractory, whereas the polarizability is only slightly reduced by refractoriness

(6) The local response can be increased by making the nerve supernormal, whereas the polarization potential is slightly reduced or unaffected during the supernormal phase

(7) The spatial spread of the two processes is different The polarization potential falls to one-third of its value in approximately 0 5 mm whereas the local response usually declines to about two thirds in that distance Under certain conditions the local response may spread very much further than the polarization potential

The latency of the nerve fibre is determined by the time relations of the local response The latency is short when the local wave has a rapid rise and fall, whereas it is long when the wave has slower time relations It is suggested that the latency of the fibre is due to the time required for the local response to reach propagating size Under certain conditions the latency may be enormously increased When this happens it is found (1) that the local response is prolonged, often by the development of a secondary hump on the falling phase, (2) that the response spreads for an unusually long distance, (3) that threshold spikes start at a distance from the cathode It is, therefore, suggested that the long latencies occur when the excitability is depressed beneath the cathode, so that the local response runs along the fibre and works up to propagating size at a short distance from the cathode

The potential at which the local response starts to propagate is increased during the relatively refractory period and decreased during the supernormal phase In a normal nerve the response starts to propagate when its amplitude is equal to about one fifth of the spike During refractoriness this large safety margin may be reduced to the point at which a propagated action potential is only slightly larger than one which just fails to propagate Under these conditions both sub and superthreshold waves of activity at first spread along the nerve in the same way and do not finally separate until they have travelled a short distance from the cathode

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The androgenic activity of ovarian grafts in castrated male rats

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[Plates 7 9]

1 Introduction

Evidence of androgenic activity on the part of the ovary has been accumulating for some years (see Parkes 1937, Lipschutz 1937) For instance, the comb of the fowl, which is insensitive to recognized female hormones but responds to androgens, undergoes development in the laying hen Moreover small amounts of androgens, detectable by capon comb tests, have been found in crude extracts of pig ovary Under conditions in which excessive luteinization of the ovary occurs, e g after irradiation or administration of pituitary or urine of pregnancy extracts, Steinach and Kun (1931) and Guyénot and others (1932, 1933 1936) found evidence of masculinization of the female guinea pig Guyénot and Wietrzykowska (1935) also described a similar condition in guinea-pigs descended from an interspecific cross (*Cavia aperea* ♂ from the Argentine × *C cobaya* ♀) Lipschutz (1933) observed occasional growth of the clitoris of the guinea-pig about a year after an operation consisting of removal of one immature ovary and reduction of the other to a minute fragment Earlier, Lipschutz (1932) had found that an ovarian graft in a castrated male guinea-pig, killed after three years, had actually effected full restoration of the prostate and seminal vesicles

Recently the production of androgens by ovarian tissue has been demonstrated in the most striking manner by Hill (Hill and Gardner 1936, Hill 1937*a*, *b*), who grafted the ovaries of litter mates into the ears of castrated male mice and obtained full restoration of the seminal vesicles in 2–3 months In a series of experiments Hill showed that such growth could not be produced by oestrone or progesterone representing the ordinary female hormones, and further that ovarian grafts made into the abdomen or elsewhere than into the ear did not show androgenic activity (Hill 1937*a*) Later he showed that the androgenic activity of the grafts could be controlled by environmental temperature (Hill 1937*b*) When mice with grafts were kept at 33° C no growth of the atrophic male accessory glands occurred, but in others kept at 22° C, active seminal vesicle growth was seen The histological appearance of the grafts is not described in detail by Hill, but they were said to contain lutein-like cells and normal and haemorrhagic follicles

It may therefore be concluded from the literature that the normal ovary is capable of secreting small amounts of androgenic substances and that under unusual or experimental conditions sufficient androgens may be secreted for their presence to be readily detectable Lipschutz (1937) emphasizes the fact that the secretion of male hormone may occur from ovaries not obviously abnormal in histological appearance

In view of the importance of Hill s experiments it was decided to extend the work to rats (Deanesly 1938) Results similar to those of Hill have been obtained from the experiments described below, and a study has been made of the histology of ovarian ear grafts showing androgenic or oestrogenic activity or both The reactions of the grafts to gonadotrophic substances have also been investigated

2 MATERIAL AND TECHNIQUE

(*a*) *Animals, operative technique, etc* The rats used were from the colony maintained at the Field Laboratories of the National Institute for Medical Research, this colony is of the Wistar strain but not continuously inbred Males were castrated as immatures at a weight of 40–60 g in such animals after castration the prostate weighs 16–20 mg and the seminal vesicles 6 mg Pieces of ovary taken from slightly younger females (30–40 g), not litter mates, were implanted in the ears either at castration or about 2 weeks later To implant the graft the skin was incised on the dorsal side of the ear (as described by Hill 1937*a*) and a little pocket was made into which the piece of ovary was pushed, no sutures were necessary All operative procedure was carried out aseptically under ether Successful grafts were readily distinguished a week or so later as vascularized swellings The proportion of grafts becoming implanted was fairly high, but not all of these survived long enough or grew to a large enough size to provide significant experimental results Rats 1–24 received grafts in March 1937, and with one exception were killed up to 6½ months afterwards other rats received grafts in August, September and October and were killed up to 4½ months later The rats were kept in a warmed animal room or on a balcony exposed to atmospheric conditions

The principal gonadotrophic substance used in attempts to stimulate the androgenic secretion of the grafts was a urine of pregnancy extract (UP 10) previously assayed on immature male and female rats (Deanesly 1935) An extract of pregnant mare serum (PMS 16) was used in 2 mg daily doses for a few rats

(*b*) *Histological technique* The grafts prostate and seminal vesicles were fixed in Bouin's fluid and were later transferred to 70 % alcohol, dissected according to the method described elsewhere (Callow and Deanesly 1935) and, except for Nos 2 and 22, weighed on a torsion balance before being embedded Serial sections at 10 μ were made of all grafts, and stained in haemalum and eosin The prostate was considered to have been stimulated if it was obviously enlarged or if its epithelium was restored and secreting (fig 4), to insure accurate diagnosis all prostates weighing less than 80 mg were histologically examined The seminal vesicles in the castrate rat enlarge in response to oestrogenic as well as to androgenic stimulation, but the two reactions are easily distinguishable microscopically (Freud 1933) These glands were therefore sectioned in all cases

3 UNSTIMULATED GRAFTS

(*a*) *Effects on the accessory organs* Tables I and II show the size of the prostate and seminal vesicles in twenty-nine rats carrying ear grafts negative results associated with grafts weighing only 4–8 mg are excluded from the tables, since sections showed that the small amount of ovarian tissue in such grafts was commonly degenerating Out of twenty-nine grafts, twenty-four listed in Table I were found to be androgenic, since the corresponding prostates seminal vesicles or both showed evidence of stimulation Actually the prostate weighs no more than 60 mg in sixteen out of twenty-four rats, while in the remaining eight rats it varies from 81 to 910 mg If the grafts are compared, it is found that those corresponding to the small prostates have an average weight of 23 mg and those corresponding to the large prostates an average of 46 mg Although this point cannot be unduly stressed—the weights of the grafts are not always proportional to the content of functional ovarian tissue—it is clear that the activity of the graft, as reflected by the accessory organs, varies with its size Moreover since the degree of androgenic activity may be very slight, no fundamental distinction is indicated between the grafts in Tables I and II

All functional grafts show oestrogenic activity, and this develops in a much shorter time than androgenic activity It was rare to find seminal

TABLE I RATS WITH UNSTIMULATED ANDROGENIC GRAFTS

	Duration of graft days					
No	Animal house	Balcony	Total	Graft mg	Prostate mg	Seminal vesicles mg
58	14	23	37	35*	46	17 +
108	37	—	37	8	29	16 –
66	50	-	50	5*	31	21 + +
54	10	43	53	24*	39	19 + +
113	65	—	65	19*	23	11 –
68	78	—	78	38*	42	28 –
29	14	76	90	48*	38	29 –
72	90	—	90	23	23	31 +
160	96	—	96	6	33	34 +
122	9	87	96	17*	81	20 –
45	14	83	97	21*	34	32 –
149	9	110	119	27	236	24 + +
110	119		119	64*	134	60 –
75	10	114	124	21	50	19 +
89	5	125	130	37*	162	20 + +
115	135		135	58	910	155 + +
159	137	—	137	13	44	20 +
35	138	—	138	27	54	44 –
22	75	84	159	—	177	31 + +
11	75	94	169	46	116	56 –
18	75	122	197	26	29	41 –
19	75	122	197	22	53	70 –
20	78	122	200	24	53	22 –
24	183	91	274	43	150	41 + +

* Graft infiltrated by leucocytes

TABLE II RATS WITH UNSTIMULATED NON ANDROGENIC GRAFTS

	Duration of graft days					
No	Animal house	Balcony	Total	Graft mg	Prostate mg	Seminal vesicles mg
144	37	—	37	29*	22	16
17	75	—	75	55*	23	17
42	14	83	97	12	42	13
53	14	75	89	21*	19	21
2	180	—	180	—†	40	60

* Graft infiltrated by leucocytes

† Graft well developed but not weighed

vesicle rudiments that were not fibrosed (cf Freud 1933) and the tables show that oestrogens stimulated the development of 60 mg seminal vesicles in which the glandular epithelium was completely inactive When the grafts became highly androgenic the developing vesicular epithelium displaced the earlier formed fibrous tissue (fig 7, Plate 7) The response of the seminal vesicles is classified as follows in the tables – indicates no appreciable proliferation of the epithelium, + indicates that the epithelium has proliferated but is not yet secreting and + + indicates a secretory epithelium (figs 5, 6, 7, Plate 7) An appreciable androgenic reaction of the seminal vesicles indicated by the sign + is much rarer than a growing prostate, suggesting that the output of male hormone by the grafts is frequently too low to affect the vesicular epithelium which requires a higher threshold (Callow and Deanesly 1935) The absence of any androgenic response in the accessory organs corresponding to the grafts listed in Table II could be explained by their histological structure (see below) Three out of the five grafts showed evidence of extensive degeneration although some ovarian tissue persisted only in rat no 2 was the graft thoroughly healthy

It may therefore be regarded as normal for ovarian ear grafts of this type to develop some androgenic activity, and it is doubtful if the latter should be attributed to the direct action of external conditions as in Hill's temperature controlled mice In the original group of eight animals—rats nos 2–24—grafted in March and kept through the summer, only the six rats under cool conditions on the balcony developed androgenic grafts Of these no 24 was kept in the animal house for 6 months and was then laparotomized and examined before being transferred to the balcony No growth of the prostate was apparent and the accessories were probably in the same condition as those of rat no 2 killed at the same time (Table II) Three months after transference to the balcony the prostate of rat no 24 was well grown (Table I)

The first few results, therefore, suggested that cool conditions favoured androgen production by the graft, but subsequent work did not confirm this hypothesis It will be seen from Table I that the most active graft, associated with a prostate of adult size, belonged to rat no 115 kept in the warm room, and excluding this, the range of variation is not very different in the two temperature groups In spite of their size and long duration the grafts of nos 18, 19 and 20 were hardly androgenic though subjected to cool conditions It still remains a possibility, however, that warmth tends to inhibit or restrict androgen production by the graft, and further experiments are now in progress The animal room was artificially heated, but its temperature varied somewhat with external conditions and the average would have been higher in the summer than in the winter months

(*b*) *The histology of the grafts* It may be assumed that any factor such as temperature affecting the graft indirectly through the pituitary of the host, or directly, would influence its histological structure, and accordingly all grafts were microscopically examined The grafts naturally varied a good deal, but it was found that their histological appearance could be correlated satisfactorily with their secretory capacity, as evidenced by the prostate and seminal vesicles There was no histological distinction between the androgenic grafts of rats kept on the balcony and of rats kept in the animal house, although the non-androgenic grafts in Table II differed in certain respects from the more active of the grafts in Table I Since the rats used in these experiments were not inbred (see Loeb 1930, Strong 1936) a large proportion of the original grafts failed to establish themselves, and others, after surviving 1–4 months, were found to be infiltrated with leucocytes * Such grafts showed degeneration of ovarian tissue to a varying extent, if this had gone far the grafts were not actively androgenic at death In some cases they had probably been more androgenic at an earlier period—for instance, the seminal vesicle epithelium occasionally showed proliferation, although it was not active at death Many grafts contained leucocytes in the stroma, but only those heavily infiltrated are recorded as such in the tables The latter include some good sized grafts with little or no androgenic activity (e g no 29, Table I)

In established grafts follicles enlarge almost to oestrous size, but there seems to be no ovulation, the ova apparently become absorbed Blood follicles are fairly common A graft containing many large cystic follicles is generally markedly oestrogenic, e g no 11, in which the seminal vesicles weigh 56 mg although their epithelium is inactive

Luteinization of the granulosa cells appears rare in unstimulated grafts (cf Goodman 1934, Pfeiffer 1937), although occasionally seen, for example in the graft of no 58, killed 37 days after the transplantation More often the granulosa cells may enlarge and acquire eosinophil cytoplasm, but it is doubtful if this is a true glandular development, or an early stage of degeneration, since the theca interna takes no part in the process, and the cells remain more or less unvascularized

In the androgenic grafts the most striking feature was irregular luteinization, the latter being used as a general term for the development of large cells of glandular appearance, with a superficial resemblance to those of normal corpora lutea Such luteal cells definitely originate from the theca interna of the follicles—in other words the grafts show typical theca luteinization (fig 14, Plate 9), which was described by Hill and Parkes (1930) in the ferret after injections of gonadotrophic substances, and has since been observed elsewhere under a variety of conditions (Selye, Collip and Thomson 1933 Guyénot and others 1933) Early stages of the process represented by follicles of all sizes with enlarged theca cells and intact granulosa, are very common later the luteinized theca cells tend to encroach on the granulosa as it becomes pycnotic, and eventually they form a solid lutein mass, often with the remains of the granulosa degenerating in the centre Thecal corpora lutea differ from normal corpora lutea in their irregular vascularization (fig 12, Plate 8, fig 17, Plate 9) and they can generally be distinguished as such even in long-standing grafts Since these "theca luteinized" cells are abundant in the more androgenic grafts, it may reasonably be inferred that they are the principal producers of the androgenic secretion

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* An outbreak of ear scabies had an adverse effect on many grafts

This conclusion is confirmed by a study of the non androgenic grafts listed in Table II Three of these grafts show evidence of leucocytic infiltration and extensive degeneration of ovarian tissue, and need not be described in detail Cells of obviously glandular appearance are rare or absent, and the stroma is dense The graft of rat no 2 which had persisted for nearly 6 months in a warm room without producing enough androgenic secretion to affect either prostate or seminal vesicles, consisted mainly of more or less cystic follicles with fairly healthy granulosa There were no corpora lutea and only rare patches of glandular cells (fig 8, Plate 8) The theca interna of the follicles in the graft remained almost entirely unluteinized The graft of no 42 resembled that of no 2 but was in a less healthy condition, parts of it showing leucocytic infiltration It contained a few patches of theca luteinized cells In these two the enlargement of the prostate as well as the seminal vesicles was due to proliferation of fibrous tissue, stimulated by the oestrogenic secretion from the graft

4 GRAFTS STIMULATED BY INJECTIONS OF GONADOTROPHIC SUBSTANCES

(*a*) *Effects on the accessory organs* With a view to obtaining further information about the conditions affecting androgen production, rats with grafts were given gonadotrophic injections of 0 4–1 0 mg daily of urine of pregnancy extract U P 10, at intervals of 6–133 days after transplantation, and killed 1–10 days after the last injection (Table III) Attempts to stimulate recently grafted tissue failed almost completely, when gonadotrophic injections were begun 6 days after transplantation and continued for 10 days, only three of twelve rats had grafts at the end of the experiment All the grafts were infiltrated by leucocytes and apparently degenerating, the only one of these listed in Table III (no 184) which weighed 32 mg, contained patches of theca lutein cells and the corresponding prostate showed histological evidence of very slight stimulation The accessory organs in the other two rats were of castrate type

At 14 or 15 days after transplantation of the graft, three rats were injected for 10 days and killed one day after the last injection It was found that in two of the rats the grafts were degenerating, while in the third (no 129 Table III), though the graft had reacted to the gonadotrophic injections, the accessory organs showed only oestrogenic stimulation There is no evidence to show whether the high proportion of degenerating grafts in these particular experiments should be attributed to the injections, but it seems to be clear that large androgenic grafts cannot be produced rapidly by this method Hill found gonadotrophic extracts useless to stimulate androgenic grafts in his mice *

In other experiments listed in Table III twenty one rats carrying grafts at least two months old were injected for 5–20 days and killed 1 14 days after the last injection Individual details of the amounts are not included since the variation in the grafts did not appear to depend on the dosage The grafts often underwent marked enlargement and hyperaemia particularly after stimulation by extract of pregnant mare serum but although androgenic they seemed to be hardly more so than control grafts of a similar age (Table I) This is best seen in fig 1 where the weights of prostates showing androgenic histological stimulation are plotted against the ages of the corresponding ovarian grafts There is no clear separation between the injected and control rats, and consequently no proof that the gonadotrophic injections have stimulated the androgen producing tissue to any appreciable extent, although it remains a possibility that this may have occurred in some cases The activity has not increased in proportion to the increase in size in the stimulated grafts (Tables I and III)

(*b*) *Histology of the grafts* The effect of gonadotrophic injections on the structure of the grafts naturally varied according to the age of the latter and their condition when injections were begun A comparison of Tables I and II and examination of fig 1 shows that the untreated series comprised adequate control material As stated above, attempts to stimulate recently implanted grafts were almost uniformly unsuccessful, rat no 129, however, which was injected for 10 days, 14 days after the transplantation was made possessed an active functional graft which, however, was not androgenic. Some development of the accessory glands had taken place but the glandular epithelium was hardly active at death. Serial sections of the graft showed that there was irregular luteinization of the granulosa cells in cystic, unovulated follicles. The graft contained a large proportion of such lutein tissue, but comparatively little luteinized thecal or luteinized interstitial cells. This suggested that, although the gonadotrophic injections had stimulated the luteinization of the granulosa, as in the immature rat ovary and in the ovarian grafts described by Goodman (1934), this type of

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* Personal communication from Professor R T Hill

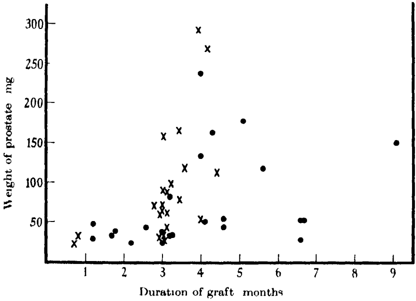

FIG. 1. Relation of prostate growth to the duration of the graft. ● In unstimulated rats, excluding no. 115, Table I. × In rats receiving gonadotrophic injections.

glandular cell did not produce androgenic secretion in appreciable amount. A study of other grafts, stimulated by urine of pregnancy extract, tended to confirm this conclusion. Most of them showed extensive luteinization and the two types of lutein cells could generally be distinguished by their appearance (figs. 16, 17, Plate 9). Grafts showing extensive granulosa luteinization, however, and a small amount of theca luteinization were no more androgenic than others with a small amount of theca luteinization, and little or no granulosa luteinization. Rats, nos. 156 and 157 from the same group, with prostates of the same size, illustrate this point. In rat no. 156 both types of glandular cells are well developed and the graft consists mainly of corpora lutea, in rat no. 157, although injected for a longer period, the graft consists of small follicles with luteinized theca and there is no obvious luteinization of granulosa cells Again, rat no 98 had a completely luteinized graft when killed, in which the luteal tissue had obviously arisen from granulosa cells and showed regular vascularization (fig 13, Plate 8) but this graft was not androgenic, merely oestrogenic

TABLE III RATS WITH GRAFTS STIMULATED BY URINE OF PREGNANCY EXTRACT* ALL ANDROGENIC EXCEPT NOS 129 AND 98

	Duration of graft days					
No	Before injection	Days injected	Total	Graft mg	Prostate mg	Seminal vesicles mg
184	6	10	22	32	21	8 –
129	14	10	25	28	31	16 –
65	61	20	91	93	159	38 + +
95	61	20	91	30	90	49 –
98	61	20	91	24	33	27 –
94	73	20	93	28	165	37 + +
32	78	20	108	37	118	51 +
82	76	20	97	86	99	29 +
86	78	13	92	28†	21	22 –
156	83	5	89	37	60	35 +
167B	83	6	90	10	64	25 –
157	83	10	94	28	61	50 –
162	83	10	94	25	89	27 + +
163	83	10	94	12	44	23 +
107B	94	7	101	25	79	30 –
41	90	20	120	29†	53	21 +
99	110	9	119	22	294	65 + +
106B	117	7	125	14	267	45 + +
77B	127	5	133	32	111	46 + +
116*	78	5	84	72	70	32 –
121*	78	5	88	14	29	10 +
137*	79	8	92	90	72	47 -

* 116, 121 137 received instead, 2 mg daily of pregnant mare serum extract
† Graft infiltrated by leucocytes
B Rats kept on the balcony

Three rats with grafts received injections of a very active extract of pregnant mare serum (Table III) beginning 78 and 79 days after grafting, in two of these the grafts were greatly enlarged when the animal was killed, but the effect on the accessory glands was not very striking The grafts showed extensive well-vascularized, granulosa luteinization, here and there, luteinized theca cells could be seen and it was probable that some theca corpora lutea persisted from before the injections were begun In spite of the relatively large amount of lutein tissue the seminal vesicles had hardly reacted

Examination of the grafts from rats which received gonadotrophic injections leaves it uncertain whether or not such treatment ever stimulates the development or function of glandular cells of thecal origin This may sometimes occur, especially where the granulosa cells are no longer sufficiently healthy to respond or where the thecal cells are already taking on a glandular function It may be stated definitely, however, as a general conclusion, that gonadotrophic extracts tend to cause luteinization of the granulosa, and that this type of lutein tissue is not actively androgenic

DISCUSSION

The present experiments have shown not only that ovarian tissue can produce an androgenic secretion (as earlier workers have reported), but also that this will occur fairly regularly when the theca interna cells enlarge and become glandular Successful ovarian grafts consist of active functional tissue including large follicles, but they differ from normal ovaries among other things in the absence of ovulation Goodman (1934) and Pfeiffer (1937) who grafted rat ovaries into the anterior chamber of the eye, both agree that such grafts do not develop corpora lutea in normal or castrate males Pfeiffer as the result of his experiments maintains that the male rat hypophysis has a low output of luteinizing hormone Fully developed granulosa failing to luteinize, commonly begins to degenerate, and under these circumstances luteinization of the theca interna cells often takes place (cf Selye, Collip and Thomson 1933) In grafts stimulated by gonadotrophic injections, luteinization of the granulosa may occur side by side with luteinization of the theca (figs 16, 17, Plate 9), but the two processes are not seen together in the same follicle Goodman (1934) found that ovarian grafts in castrate males could be luteinized by urine of pregnancy extract, although there is no suggestion that these grafts were androgenic On the other hand it is interesting to note that Guyénot and his collaborators (1933) found marked theca luteinization in the intact ovaries of guinea-pigs which showed masculinizing effects after pituitary injections

Mossman (1937) who has studied the theca interna cells in *Geomys* and elsewhere stresses the fact that they are glandular but distinct from the true corpus luteum cells and closely connected with the interstitial tissue of the stroma

In these experiments theca luteinization seems to occur independently of the temperature at which the rat is kept The fact that Hill (1937*a*, *b*) was able to control the androgenic secretion of ovarian grafts in mice by temperature regulation suggests that he thereby influenced the histological development of the graft Cold temperatures might well stimulate theca luteinization, possibly through the pituitary, or possibly merely through having an adverse effect on the granulosa cells Incidentally microphotographs of ovarian grafts in a mouse testis (Strong 1936, figs A & B) seem to show theca luteinization, although this is not referred to by the author

Most of the rat grafts were only slightly androgenic, and even when the prostate was enlarged considerably the seminal vesicles were relatively small or of a non glandular type (fig 5, Plate 7) This discrepancy between prostate and seminal vesicle growth suggests that the graft is not producing testosterone, the naturally occurring androgen of the testis, but some other less active substance similar to androsterone, which also causes unequal development of the two accessory glands (Callow and Deanesly 1935) Hill and Strong (1938), for different reasons also conclude that the androgen secreted by the ovarian graft is not testosterone

The pregnant mare serum extract used was Antex', kindly provided by Mr A Kongsted, Løvens Kemiske Fabrik, Copenhagen

SUMMARY

1 Ovarian grafts were made into the ears of immature castrated male rats

2 All functional grafts showed oestrogenic activity, detectable by the fibrosis of the seminal vesicles

3 Most functional grafts especially those established for three months or more, showed androgenic activity causing secretion and growth in the prostate and occasionally in the seminal vesicles One rat with a graft 135 days old, had a prostate of full adult size, though the seminal vesicles were not correspondingly large The slighter response of the vesicular epithelium as compared with that of the prostate suggests that the androgenic substance produced by the grafts is not testosterone

4 Androgenic activity does not depend on the temperature at which the rat is kept, it is associated with luteinization of the theca interna of the follicles which occurs commonly in established grafts

5 Gonadotrophic extracts which cause extensive luteinization of the granulosa do not appreciably affect the androgenic activity of the graft

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DESCRIPTION OF PLATES

Plate 7

Sections through prostate and seminal vesicles from castrate rats Figs 2 and 3 prostate and seminal vesicle from castrate control, figs 4 7 prostate and seminal vesicles from rats with ovarian grafts

FIG 2 Section through prostate of an immature castrate rat showing small tubules and inactive dense staining epithelium × 116

FIG 3 Section through seminal vesicle of immature castrate rat showing rudimentary condition × 24

FIG 4 No 20 (Table I) Section through prostate where slight androgenic stimulation has led to an increase in the size of the tubules and restoration of the epithelium although the whole gland is not greatly enlarged × 116

FIG 5 No 2 (Table II) Section through seminal vesicle showing fibrosis due to oestrogenic stimulation and inactive epithelium × 24

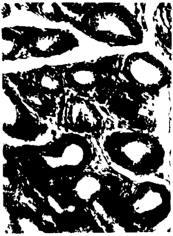

Fig. 2

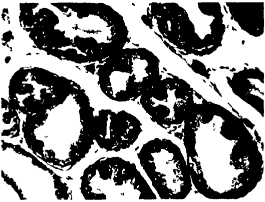

Fig. 4

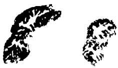

Fig. 3

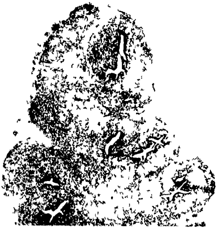

Fig. 5

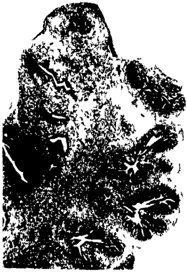

Fig. 6

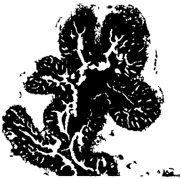

Fig. 7

(Facing p. 134)

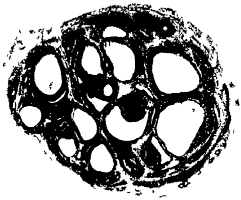

Fig 8

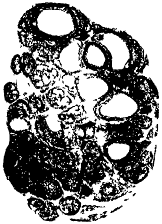

Fig 9

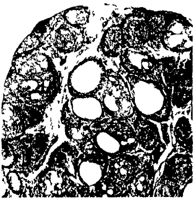

Fig 10

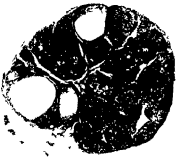

Fig 11

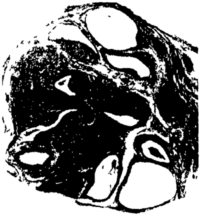

Fig 12

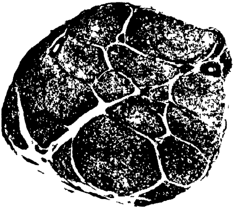

Fig 13

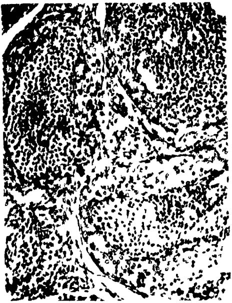

Fig 14

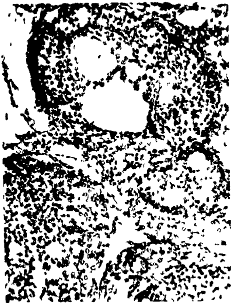

Fig 15

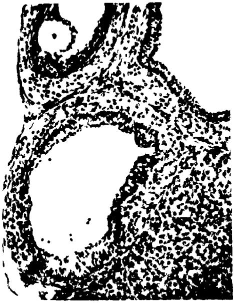

Fig 16

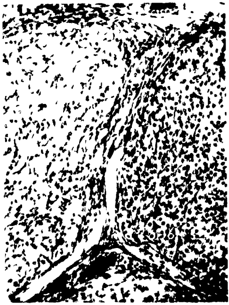

Fig 17

FIG 6 No 22 (Table I) Section through seminal vesicle showing oestrogenic and androgenic stimulation epithelium proliferating but not yet secreting (+ stage) × 24

FIG 7 No 24 (Table I) Section through a seminal vesicle with actively secreting epithelium (+ + stage) induced by androgenic stimulation × 24

Plate 8

Sections through ovarian grafts × 30

FIG 8 No 2 (Table II) Oestrogenic non androgenic graft 180 days old showing cystic follicles and few glandular cells

FIG 9 No 22 (Table I) Androgenic graft 152 days old showing both cystic and solid follicles and abundant theca luteinization

FIG 10 No 115 (Table I) The most highly androgenic graft of the series 135 days old showing numerous small anovular follicles with luteinized theca interna

FIG 11 No 106 (Table III) Androgenic graft 125 days old from a rat injected with urine of pregnancy extract for a week before death Luteinization mainly of thecal origin without well defined corpora lutea It is doubtful if this graft was much affected by the gonadotrophic injections cf figs 10 and 13

FIG 12 No 11 (Table I) Androgenic graft 169 days old showing irregular corpora lutea probably of thecal origin

FIG 13 No 98 (Table III) Graft 91 days old and hardly androgenic Stimulation with gonadotrophic injections for 20 days has led to the formation of corpora lutea of normal appearance derived from granulosa cells

Plate 9

Sections through androgenic ovarian grafts × 142

FIG 14 No 22 (Table I) Part of fig 9 theca luteinization

FIG 15 No 115 (Table I) Part of fig 10 theca luteinization

FIGS 16 17 No 156 (Table III) Graft 89 days old stimulated by gonadotrophic injections Fig 16 shows follicles with luteinized theca and, next to them, part of a corpus luteum formed normally from granulosa cells Fig 17 shows on the right, a similar corpus luteum and on the left an irregular corpus luteum which appears to be composed of luteinized theca cells

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The heat of shortening and the dynamic constants of muscle

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The hope was recently expressed (Hill 1937, p 116) that with the development of a more accurate and rapid technique for muscle heat measurement, a much more consistent picture might emerge of the energy relations of muscles shortening (or lengthening) and doing positive (or negative) work This hope has been realized, and some astonishingly simple and accurate relations have been found relations moreover, which (among other things) determine the effect of load on speed of shortening, allow the form of the isometric contraction to be predicted and are the basis of the so-called visco-elasticity" of skeletal muscle This paper is divided into three parts In Part I further developments of the technique are described everything has depended on the technique, so no apology is needed for a rather full description of it and of the precautions necessary In Part II the results themselves are described and discussed In Part III the "visco elastic ' properties of active muscle are shown to be a consequence of the properties described in Part II

PART I METHODS OF MEASURING THE ENERGY LIBERATION OF MUSCLE

(*a*) *Galvanometer system* In the earlier stages of the present work a single moving coil galvanometer of rather short period (0 2–0 3 sec) was employed, as described in the previous paper (Hill 1937, pp 115 and 131) Such a galvanometer, however, was far slower than the thermopile, and the interpretation of records obtained by it was difficult, or impossible, without laborious numerical analysis The galvanometer could not be made quicker without reducing its sensitivity too much, amplification therefore was necessary if recording was to be quick enough

Now the rise of temperature which it was particularly desired to measure was that associated with shortening, which in a muscle 3 cm long allowed to shorten 5 mm. proved to be of the order of $1–2 \times 10^{-3}$ °C. The thermopiles gave about 1500 μV per 1° C, so the change to be observed was of the order of 1·5–3 μV. If this was to be determined to within 4 %, it was necessary to avoid disturbances greater than 0·06–0·12 μV.

It is frequently asked why amplification by thermionic valves is not used. Dr Otto Schmitt of St Louis advises me that the present limit of direct measurement with valves, even for the relatively slow processes involved in this work, is of the order of 0·3 μV, so the direct method would not be nearly accurate enough. Indirect methods might be used, involving some special arrangement designed essentially to transform up the small potential difference, in the low impedance of the thermopile, to a larger potential difference, in an impedance more comparable with the input impedance of a valve. This impedance matching might be carried out in several ways, the simplest of which would involve frequency or amplitude modulation of a carrier oscillation and subsequent amplification at high audio- or radio-frequency. Such a technique, however, although in principle about ten times better than present galvanometric methods, is not at present available and would have to be developed.

The alternative of building a finer thermopile with many more junctions, and using direct amplification might have a better chance of success. Since a valve has a very high input impedance a high resistance thermopile could be used. If it were possible to build a thermopile with 500 couples, not by soldering where the limit has already been nearly reached, but perhaps by electrodeposition, or sputtering of the active metals upon a thin former and subsequently slicing the film into a thin continuous spiral, the desired sensitivity could be achieved. The construction, however, of so fine a thermopile would require much time and effort, without any certainty of ultimate success. One may conclude, therefore, that valve amplification may indeed be applicable to measurements of muscle heat, but not with present instruments or methods (see also p. 155 below).

It was decided, therefore, to fall back on the older method of amplifying the deflection of the galvanometer by photo-electric means, the light returning from the mirror falling upon a differential photo-cell and producing a current which deflected a second galvanometer of short period (Hill 1932, 1934*a*, *b*, Downing and Hill 1935). The secondary galvanometer gave 20–25 times the deflexion of the primary galvanometer. The resulting sensitivity was unnecessarily high, and an adjustable fraction of the current from the photo-cell was "fed back" into the primary galvanometer in order to make it quicker, more stable and less sensitive (cf. Watton 1935, Appendix by Hill). By this the sensitivity could be set to the value required, and any unnecessary sensitivity was not wasted but used to make the system quicker and more stable

The secondary galvanometer, constructed by Mr A C Downing was of the type recently described (Hill 1937, Appendix I), but with much stiffer suspension (above and below) and so a very short period

Period 0 053 sec
Internal resistance 120 ohms
External resistance for critical damping 500 ohms
Coil dimensions $24 \times 1 \times 1$ mm , weight 54 mg
Wire $30\,\mu$ copper 100 turns
Suspension $16\,\mu$ phosphor bronze, 5 mm at each end
Mirror $3\frac{1}{2} \times 1\frac{1}{2}$ mm
Sensitivity at 2 m distance, nearly damped 1 mm $= 3\ 4 \times 10^{-8}$ amp

It was mounted on a Julius suspension and was perfectly steady Records were made from it on a camera drum moving at 8 or 13 cm /sec The lower half of the light beam was intercepted by mirrors and observed simultaneously on a scale

The primary galvanometer, constructed by Mr Downing, was of the same type but suited to a lower resistance circuit and of longer period

Period 0 0965 sec
Internal resistance 44 ohms
External resistance for critical damping 70 ohms
Coil dimensions $24 \times 1\ 3 \times 0\ 5$ mm
Wire $40\,\mu$ silver, 50 turns
Suspension $16\,\mu$ phosphor bronze, 12 mm at each end
Mirror $3\frac{1}{2} \times 2$ mm
Sensitivity at 2 m distance 1 mm $= 2\ 8 \times 10^{-8}$ amp

It was adjusted for the greatest possible stability by aligning the coil with the suspension With the high amplification employed, the stability was at first insufficient (at the top of a building in London), and the primary galvanometer, lamp, lenses and photo cell were mounted together on a teak stand floating on mercury in a trough 78×40 cm in size (cf Downing, Gerard and Hill 1926) A little thick oil on the top of the mercury helped to damp oscillations With appropriate feed-back of the photo-current the stability was now sufficient, and when the laboratory was quiet the amplified deflexion could be read on a photographic record to 0 1 mm

A 12 V, 36 W motor head lamp was used with large accumulators to provide the illumination A large condensing lens focused the light on the galvanometer mirror, from which it was focused again on the two halves of a large photo-cell of the rectifying type ("GMBH electro-cell") arranged differentially (in opposition in parallel) The platform on which galvanometer, lamp, photo cell, etc floated was heavily loaded The photo-current was led to the pair of resistance boxes shown in fig 1 by which the feed-back was regulated R_1 was usually kept at 1000 ohms the impedance of the photo cell was high so this did not matter, and it was undesirable to lose sensitivity by short-circuiting the thermopile with a lower resistance The feed back was regulated by adjusting R_2, as shown by the example in Table I

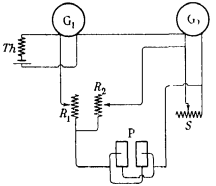

FIG 1 Circuit of coupled galvanometers G_1 primary throwing light on to photo cell G_2 secondary P, rectifying photo cell two halves arranged in opposition in parallel R_1 and R_2 resistances for adjusting the feed back to G_1 S, shunt to G_2 for adjusting damping Th, thermopile connected to G_1

TABLE I DEFLEXION OF SECONDARY GALVANOMETER G_2 FOR 1 μA IN PRIMARY GALVANOMETER G_1

R_1 (ohms)	0	1000	1000	1000	1000	1000
R_2 (ohms)	1000	1000	300	100	50	0
Deflexion (mm)	28	51	104	230	350	800
Period of G_1 (msec)	18	24	35	52	63	96

The period was calculated from that observed without ' feed back", assuming it to be proportional to the square root of the sensitivity

It was found that the damping of the whole system could be sufficiently adjusted by the shunt S of G_2 Critical damping is not wanted It is better, indeed, for quickness to use the system rather underdamped, for the heat production of muscle occurs relatively slowly, and no overshooting occurs For working at 0° C where the heat production is rather small, R_2 was usually about 130 ohms and the period of G_1 about 50 msec This gave sufficient stability, and the amplified deflexion to a constant current was nearly complete in 35–40 msec For higher temperatures R_2, and the feed-back, could be made greater, and the period of G_1 reduced to 35–40 msec , with an amplified deflexion nearly complete in 30 msec The system, therefore, was fast enough to allow the thermopile current to be recorded with very little lag For most purposes a full numerical analysis of records was not needed, which greatly simplified and quickened the research

(*b*) *Thermopile* The three instruments particularly used were similar to the one described before (Hill 1937) All were "protected" against the errors due to movement by having a sufficient set of dummy couples, or of similar constantan wires, at their ends The "protection" seems to be very effective For details see Table II below under "Calibration"

Thermopile P 2 had thirty-four constantan-manganin couples in the "protected" part It was large, it was intended for the sartorii of large Hungarian frogs, and for reasons referred to below under "Muscle" these large muscles were discarded in favour of very small ones from English frogs This thermopile, therefore, was replaced by a smaller one It gave 1286μV per 1° C

Thermopile P 4 had twenty-eight protected couples of constantan iron, sixteen protecting couples at the "thread" end, four protecting couples at the "clamp" end, and a total thickness of 55μ It gave 1415μV per 1° C Having iron instead of manganin, it had a higher thermo e m f and a lower resistance It is short—total length, including electrodes, 20 mm — and suited any frog's sartorius, however small The insulation was usually very good, in spite of its thinness If leaks appeared, the thermopile was dried, thinly painted with very dilute bakelite varnish ("yacht varnish"), dried under a lamp and the dried varnish scraped off The high insulating properties of the mica were in this way restored without thickening it The insulation was so good although only 20μ thick, that condenser discharges of 400 V, 0 03 μF, for the purpose of "control heating", gave momentary disturbances only of the order of 1 mm Stimuli showed no disturbance at all unless the air in the room was damp This instrument was used in most of the experiments (Dec 1937–May 1938)

Thermopile P 5 shown in fig 2 has forty-two protected couples, is about 45μ thick, and gives 1628μV per 1° C Being of constantan-manganin, and rather thinner, it has a higher resistance and is actually rather less sensitive than P 4 It is very flat and so designed that a small frog's sartorius pair lies, and moves, very accurately in the groove (4 mm wide) containing the hot junctions Mechanically, and for quickness, it is the best instrument hitherto constructed It has the same good insulation but only 16μ thick, as P 4 It was used in the later experiments

The quickness of response of thermopile P 5 to a sudden rise of temperature of the muscle on it, when connected to the coupled galvanometer system described above, is shown by curve *E* in fig 3 For comparison previous ' heating control" curves are given, as described in the legend Curve *A*, from the original paper by Hill and Hartree (1920*a*), rises so slowly that it had to be plotted on one-third the time scale The increase in speed, illustrated in fig 3, has made possible the experiments described

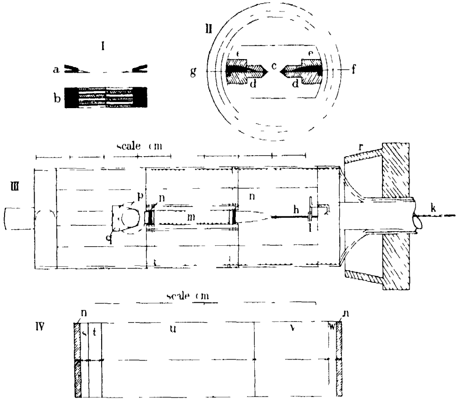

FIG 2 Thermopile P 5 (A C Downing) I and II to show construction and mounting of thermopile element III, complete instrument with muscle IV enlarged diagram of central part of thermopile element I *a* end view *b*, side view, of manganin constantan couples with loaded' cold junctions Alternate cold junctions raised to avoid short circuit II *c*, end view of thermopile element between insulated brass flanges *dd* and bakelite *ee* 4 mm exposed for contact with muscles *f*, stimulating leads *g*, thermopile leads III *h* thread to chain *k* and muscle *m* on thermopile *l* silver ring to guide thread *n* platinum electrodes *p*, pelvic bone and acetabula in clamp *q* *r* tapered brass block to carry insulated brass cover holding 105 c c of Ringer s fluid which is stirred by bubbling oxygen Before observation the fluid is removed IV central portion of element, 23 6 mm long, 45μ thick Left to right *n* electrode *s* mica alone *t*, three insulated constantan dummies *u*, forty two insulated manganin constantan couples, *v* twenty insulated constantan dummies allowing a 29% "protected ' shortening of muscle *w*, mica alone *n*, electrode Resistance 68 ohms sensitivity 1628μV/1° C The couples are of 44 s w g (81μ) wire, rolled to 12μ after hard soldering The cold junctions are loaded" with soft solder for 4 mm outwards to slow their warming up by conduction

in Part II the adoption of the "protected" thermopile has made them accurate

(c) *Calibration* In the present investigation heat and work had to be directly compared, so calibration of heat deflexions in absolute units of energy had to be as accurate as possible The method hitherto employed had been to liberate a known amount of energy electrically in the muscle, in its usual position on the thermopile This could not be very exact, since the portion of muscle heated had to be cut off and weighed, and the heating near the electrodes could not be very uniform It was found also

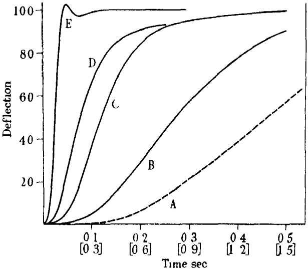

Fig 3 To illustrate improvement in speed of myothermic recording Heating controls *A* 0 1 sec heating Hill and Hartree (1920*a*) lower time scale *B* to *E* upper time scale *B* Hartree (1933) 0 05 sec heating thermopile W H 2 (Table II), moving magnet galvanometer *C*, ditto, Moll (0 19 sec) 'micro' galvanometer *D* Hill (1937 unpublished) instantaneous heating, thermopile P 2, Hungarian frog, 0 22 sec galvanometer *E* (present research) instantaneous heating, thermopile P 5, English frog coupled galvanometers

that the same amount of electrical energy in different forms (e g in single condenser discharges of different voltage and capacity, or in repetitive discharges) did not give precisely the same readings probably owing to more or less of the energy being liberated near the electrodes with the different forms of discharge This direct method of calibration had the advantage that it made automatic allowance for heat capacity and heat loss in the instrument The present thermopiles, however, are so thin that their heat capacity is very small, and the deflexions are so rapid that there is no time for any significant heat loss. It was more accurate therefore—and much simpler—to calibrate them once and for all by direct observation of the e.m.f. per 1° C. difference of temperature between hot and cold junctions, and to allow for the heat capacity in each case.

The e.m.f. per 1° C. was determined by connecting the thermopile, through a high resistance to a quick galvanometer (period 0.2 sec.), and transferring it rapidly between two baths from one temperature to another. The temperatures were read with a standard thermometer, the transference took only a second, and the galvanometer gave an immediate reading. The thermopile is so thin that its "hot" junctions take the new temperature within a few milliseconds, while its "cold" junctions are so deeply embedded under metal and insulator that they are not affected for several seconds. The deflexions are calibrated by introducing a known e.m.f. into the circuit, and very consistent readings are obtained. Details of the various thermopiles are given in Table II.

TABLE II

Thermopile	Couples	Thickness μ	Resistance ohms	e.m.f. $\mu V/1°C$	Remarks
W.H. 1	88 const. iron	210	22.5	3640	—
W.H. 2	66 const. iron	240	23.7	2775	—
P. 1	92 const. iron	125	61	4200	Whole
	64 const. iron	125	45	3100	Protected
P. 2	54 const. mang.	55	69	1997	Whole
	34 const. mang.	55	44	1291	Protected
P. 3	52 const. mang.	70	84	2010	Whole
	32 const. mang.	70	52	1228	Protected
P. 4	48 const. iron	55	55	2445	Whole
	28 const. iron	55	32	1415	Protected
P. 5	42 const. mang.	45	68	1628	Protected

The W.H. thermopiles are those used by Hartree in his latest researches (Hartree 1932, 1933). *The P. thermopiles are "protected" ones, P. 1 being the instrument recently described* (Hill 1937).

The allowance for the heat capacity of the thermopile is illustrated in the following example.

Thermopile P. 5, 12μ of wire, 33μ of mica and bakelite. $\rho c = (0.85 \times 12 + 0.63 \times 33)/45 = 0.69$. ρc for muscle $= 0.92$. Hence 45μ of thermopile is equivalent in heat capacity to 34μ of muscle. Muscle pair, 90 mg., 3 cm. long, 3½ mm. wide, average thickness 820μ. Thus 1° temperature rise of muscle gives $820/854 = 0.96°$ temperature rise of thermopile after equilibration. Hence 1° C. of muscle gives $0.96 \times 1628 = 1564\mu V$ and 1 cal./g. of muscle gives $1564/0.88 = 1778\mu V$.

In each experiment, and usually on each sheet of records, the sensitivity of the system was measured by introducing $\pm 10\mu V$ in the galvanometer-thermopile circuit If 30 mm deflexion $= 10\mu V$ 1 mm $= 0\ 333\mu V =$ (in the above example) $0\ 187 \times 10^{-3}$ cal /g This was a usual sensitivity for 0° C For higher temperatures one half to one-third of this was sufficient The records were read to $\frac{1}{10}$ mm For comparison with the work, which is measured in g cm, the heat in cal /g is multiplied by 42,600 × (weight of muscle) The muscle was cut off at its tendons and weighed at the end of an experiment

In the method of calibration adopted it is necessary that the thermo e m f per 1° C should be the same at the temperature of the experiment as at that of calibration This was tested with a manganin constantan couple and found to be the case

(*d*) *Mechanical arrangements* For an investigation of the present kind the use of a thread to connect muscle to lever is objectionable, because of its extensibility The muscle shortens more than intended, it does work in stretching the thread and this work appears as heat in relaxation A wire is better, but it is difficult to make a wire, which has to be manipulated, remain straight, and a bent wire stretches by straightening and is as bad as a thread Fine chains, therefore were used for all connexions Chains intended for jewellery can be bought at Woolworth's Stores for a few pence, and some of these are excellent They are only half as extensible as the best thread in the range of loads considered, they show no after-extension as a thread does they are perfectly flexible they hang quite straight, and they can be adjusted precisely to be just tight The best chain found consisted of small plane elliptical links, long axis 2 mm, short axis 1 mm, of chromium plated brass wire weighing about 16 mg /cm length

It is necessary, for accurate work, to be sure of the initial tension of the muscle A light isotonic lever was constructed, on which the muscle pulled twenty two times as far out as the load To avoid friction the lever was mounted on miniature ball bearings (G M B) The load on this lever determined the initial tension It was connected by a chain to the main isotonic lever, or to the isometric lever, above it It had a thin bamboo pointer which was used to record movement on a smoked drum

The main isotonic lever was of aluminium strip and was mounted on ball-bearings It provided the after-load The weight in its pan was 18 3 times the force it produced on the muscle Its stand had two adjustable screw stops, one at each end, the first to hold the after-load till shortening began, the second to adjust the amount of shortening allowed It was equipped with two electromagnets, one for holding the lever up, i e in the initial position, one for holding it down, i e in the final position The magnets were connected to the lever by threads, or later by chains. The first one maintained the contraction isometric until it was released by opening a key, when the muscle was able to shorten isotonically through the distance required. The moment of release was determined, in relation to the beginning and end of stimulation by a third arm on Lucas's revolving contact breaker. If this magnet was not used the contraction was isotonic (after-loaded) from the start. The other magnet was actuated, when required, by the contact of the lever with the second stop. By it the main weight could be held up, and not lowered by the muscle in relaxation: the muscle, in that case, relaxed under the initial load only.

The isometric lever was adjusted by a separate rack and pinion, mounted on the same stand as the rest, and was disengaged when the main isotonic lever was in use. The chains and the isometric lever together permitted 0.93 mm. shortening when the muscle developed a force of 100 g.

The whole arrangement was mounted on a Palmer screw stand by which it could be raised or lowered precisely as required.

It was important to make sure that, under the conditions of its use, the system of levers employed developed no considerable kinetic energy. Otherwise the work done by the muscle would be greater than that calculated from the load and the shortening. This was tested as follows. At time t sec. let y cm. be the displacement from its zero position of the point of attachment of the muscle to the lever, and let P grams weight be the static load on the muscle due to the weights employed. Let $\frac{1}{2}M(dy/dt)^2$ be the kinetic energy of the whole moving system, where M is its "equivalent mass" and has to be determined. Let us suddenly release the chain from the muscle (at $t = 0$ and $y = 0$) and record, on a rapidly moving drum, what happens. The work done by the weights is Pgy ergs, and if the bearings be assumed frictionless—as they nearly are—this is transformed into kinetic energy. Hence,

$$\tfrac{1}{2}M(dy/dt)^2 = Pgy$$

or, on integration
$$y = \tfrac{1}{2}Pgt^2/M.$$

Experiments showed that $\sqrt{y}/t$ was constant, from which M could be determined. Its actual value was about 1 g. This does not include the mass of the chain (about 1 g.) or the equivalent mass of the weights in the pans. The latter can be calculated: for example, 100 g. on the "after" lever is equivalent to $100/(18.3)^2 = 0.3$ g.; 100 g. on the "initial" lever to $100/22^2 = 0.2$ g.

Let us see what effect this has. High velocities occur only with small loads. At 0° C. the highest velocity of shortening observed, with a small load (total equivalent mass, say, 2 5 g), was about 4 cm /sec The kinetic energy is $\frac{1}{2} \times 2\ 5 \times 4^2 = 20$ ergs $= 0\ 020$ g cm This is quite negligible compared with the energy, or work, liberated by the muscle At 20° C, with a rather larger load, equivalent mass (say) 3 0 g a higher velocity, say 10 cm /sec , might be reached the kinetic energy would be $\frac{1}{2} \times 3 \times 10^2 = 150$ ergs $= 0\ 153$ g cm this is still negligible compared with the energy liberated by the muscle With heavier weights on the levers, the equivalent mass is rather greater with 500 g on the "main" lever and 50 g on the "initial" lever it would be 3 6 g , but with this, even at a higher temperature, the velocity of shortening would be low, and the kinetic energy would remain negligible For the muscles employed, therefore and the velocities they attained, the lever system was nearly enough "isotonic", it discharged its role of maintaining a constant pull on the muscle, its kinetic energy was negligible

The isometric lever and the chain were not quite as inextensible as could be wished but a long connexion is inevitable when the muscle is mounted on a thermopile, and the complication of an optical lever and photographic recording was to be avoided with so much else to attend to Moreover it will be shown below that the elastic portion of the muscle itself must be stretched 2–3 mm in a maximal tetanus which is several times as much as the stretch of lever and chain Work is done by the muscle in stretching lever and chain, e g , for 100 g tension developed, assuming the extension given above and normal elasticity, $\frac{1}{2} \times 100 \times 0\ 093 = 4\ 65$ g cm This appears in the muscle as heat during relaxation it would warm a muscle of 120 mg about 10^{-3}° C it provides a considerable part of the relaxation heat observed after an isometric contraction

(e) *The muscle* At first it was intended to use the rather large sartorii of Hungarian frogs with thermopile P 2 A pair of these had an average weight of 500 mg an average length of 45 mm , and each had an average thickness of about 1 mm It was found, however, that irregularities of the heat records occurred in contraction, and particularly in relaxation, which could be ascribed to no other cause than non-uniformity of the heat production and of the speed of relaxation The muscles were prepared as carefully as possible but still the irregularities persisted To get rid of them, either a muscle which contracted more uniformly and was less affected by manipulation, or a much thinner one in which thermal equilibration was quicker, was needed For both reasons recourse was had to the sartorii of small English frogs, averaging in weight about 80 mg the pair, in length 32 mm , in thickness 0 35 mm

Since the time taken in thermal equilibration depends on the square of the thickness, it is diminished to one-eighth by using the thinner muscles, and the sartorii of English frogs (which also can be obtained in better condition) seem to be less affected by experimental procedure. Being thinner, moreover, the initial soaking with Ringer's solution, and the oxygen supply, are more effective. A final advantage is that, whereas the connective tissue covering (epimysium) is thick and strong in Hungarian sartorii, it is thin and almost imperceptible in English ones. This connective tissue slows conduction of heat to the thermopile, and adds to its heat capacity. It is dangerous to try to dissect it away for there is risk of injury to the surface fibres, and if these are made inexcitable, being (say) 50μ thick, they will add greatly to the heat capacity of the thermopile and render conduction much slower. Even a single layer of injured fibres, on each face of a rapid thermopile such as P. 5 with a heat capacity equal to only 34μ of muscle would slow it in the ratio of $(134/34)^2$, i.e. about 15 times: and a layer of injured fibres by liberating potassium ions would be likely to affect the fibres beneath it, and so lead to even worse results. For exact registration of the time relations of its heat production a muscle must be quite uninjured at its surface. The sartorius of a small English frog is well adapted to myothermic experiments: but very few muscles are. For accurate work a muscle must be a regular band of tissue, very thin, contracting and shortening straight and uniformly, and quite uninjured in its preparation. Otherwise, with the present very sensitive instruments anomalous or absurd results may be obtained.

The thermopiles have been made even quicker than predicted in the previous paper by the use of the thin muscles. The curve in fig. 1*a* (Hill 1937) was calculated for a thermopile of equivalent half thickness $b = 0.0014$ cm., with a muscle of thickness $a = 0.1$ cm. on each face. With thermopile P. 5, $b = 0.0017$ cm., a pair of muscles of 80 mg., 30 mm. long and 3½ mm. wide has $a = 0.04$ cm. For $a/b = 0.04/0.0017 = 23$ the following performance can be calculated:

% of full e.m.f.	60	65	70	75	80	85	90	95	97½
Time (msec.)	3.2	4.4	6.0	8.2	12	19	35	84	150

This assumes no heat loss by conduction, etc., and no warming of the cold junctions of the thermopile. Actually such changes, though small, are not quite negligible, and their effect is to make the later stages of temperature equilibration apparently rather more rapid.

The thermopile is now about as thin as it seems possible—or even useful—to make it. By employing an even thinner muscle the thermopile response could be quickened still more. It is doubtful, however, whether any significant advantage would be gained by increasing the nominal speed of the thermopile, for it is impossible to eliminate altogether the layer of fluid between muscle and thermopile, and if this were as little even as 5μ thick on each face it would, by increasing the heat capacity from the equivalent of 34μ of muscle to one of 47μ, slow the attainment of temperature equilibrium in the ratio of $(34/47)^2$, i e to about one half Moreover, as shown above, the connective tissue on the surface of the muscle must provide a certain delay In small active young frogs, preferably females, this connective tissue layer is thinner but it cannot be avoided altogether It is doubtful, therefore whether a thinner thermopile would give any significantly better results

It might be suggested that a single thermocouple stuck right into the muscle, as in the experiments on a frog's gastrocnemius (Hill 1931 *a* Cattell 1932), would be quicker than a thermopile It would in fact be far slower, even if the small e m f of a single couple could be recorded rapidly enough for it could not fail to injure several fibres around it, and these by liberating potassium would affect the excitability of others, and the couple, however thin itself, would be surrounded by a relatively large region of inert tissue This would not be thick enough to have much effect on the magnitude of the final reading, but it would greatly slow its attainment The present thermopile is as thin as a single muscle fibre, and no arrangement involving thermal conduction through inexcitable fibres can compete with it

For similar reasons the surface of the muscle and the surface of the thermopile must be very plane, and no muscle with a belly" on it could be used When the muscle develops a tension there must be no tendency to pull away from the thermopile or to suck in fluid to fill up hollows Such hollows would greatly hinder conduction The muscle should lie as straight and as uniformly as possible it should move backward and forward as though in guides The ring *l* (fig 2) helps to keep the muscle true in its movement The acetabula are slightly off the middle line of the muscle, and it is best to fit the clamp slightly to one side on its rod to make the muscle lie true in its groove The screws of the clamp allow the bone to be adjusted to and fro so that the muscles are equally in contact with the two faces Such precautions are not insignificant they are of major importance for accurate results Hartree in his last paper (1933) insisted on their necessity, and that is increased by the use of the present thin thermopiles In some of the earlier experiments the photographic records of the heat were very sharp, showing by obvious angles the moments when shortening began or ended, or when the stimulus ceased

In others, unaccountably then, this sharpness did not appear In the former the thermal contact of active muscle with thermopile must have been good, in the latter poor Once it was realized that such precautions as are detailed above must be and can be taken, all the records have been sharp It is possible to see on the heat record, within 0 01 sec , the moment at which shortening is permitted and the extra heat associated with shortening begins

The dissection of the muscle is important, and no unnecessary bits of tissue should be left attached to it The pelvic bone should be cleared of other muscle, so that the sheet of tendon to the sartorius is clearly seen If the muscle does not lie straight and flat enough on the thermopile it had better be discarded for the results will be suspect It should be soaked in position on the thermopile, preferably loose so that Ringer's solution can get at both sides of it, for several hours before use, so as to avoid the inexcitability investigated by Duliere and Horton and by Horton (1929, 1930) If it begins to fail the experiment should be stopped and the muscle soaked again, or discarded Otherwise it may be contracting in parts, and anomalous results will occur Before the Ringer's solution is withdrawn *the muscle should be tightened to press out fluid from between* it and the thermopile and the solution should be sucked out slowly so as to draw away unnecessary fluid by capillarity Then before records are begun several contractions should be allowed, to enable the muscle to come into regular and uniform mechanical and thermal contact with the thermopile Other such precautions will occur to the experimenter, who will realize that without rather perfect thermal contact between muscle and thermopile the results of laborious experiments and calculations may be largely wasted

(*f*) *The effect of a non contracting layer of muscle on the transfer of heat to the thermopile* We have referred above to the anomalies which may be produced in the heat records by a layer of non contracting or imperfectly contracting muscle The two most serious cases and others are intermediate between them, are

(i) a layer of inert material, fluid, connective tissue, or inexcitable fibres, between muscle and thermopile,

(ii) a layer of inexcitable fibres on the outside, where injury may have occurred in dissection

In both cases the deflexion is reduced, but the more important effect is on the apparent time at which the heat is set free

(i) Let a pair of muscles, each of thickness a, have each an *inner* non-contracting layer of thickness b' in contact with a thermopile of thickness $2b$ Then in the equations of the previous paper (Hill 1937) a is to be replaced by $(a-b')$, b by $(b+b')$ The effect can be seen approximately at once, viz to make the apparent thickness of the thermopile $2(b+b')$ instead of $2b$, which would slow the rise of temperature of the hot junctions in the ratio of $b^2/(b+b')^2$ Now b is very small, e g 0 0017 cm for thermopile P 5, so that if a layer 0 01 cm thick (only about two fibres) failed to respond the early temperature rise of the thermopile would be slowed $(0\ 0117/0\ 0017)^2 = 21$ times A considerable delayed heat production would apparently result For example, as a rough estimate in a 0 2 sec tetanus at 25° C, in which the initial heat is really all liberated in about 0 3 sec, some 50 % of that heat would appear to be delayed At 0° C, where initial heat is produced far more slowly the error would not be so obvious There is no way, however, of avoiding it except to ensure that the surface of the muscle contains normal active fibres in close thermal contact with the thermopile

(ii) For the case of a muscle of which a thin *outer* layer does not contract, let us take contracting muscle and thermopile together and call their thickness b, and let the thickness of the outer non-contracting layer be a Then the analysis of the previous paper (p 119) is applicable We suppose b to be warmed suddenly at $t = 0$ to temperature y_0 The temperature of the thermopile (at $x = 0$) at subsequent times is

$$\sum_{n=-\infty}^{n=+\infty} \frac{y_0}{2\sqrt{(k\pi t)}} \int_{-b}^{+b} e^{-\{\xi-2n(a+b)\}^2/4kt}\, d\xi$$

Putting $a/b = \gamma$, and transforming, this becomes

$y_0[1-$the sum of the areas of the probability integral* between $0\ 5b/\sqrt{(kt)}$ and $(0\ 5+\gamma)\, b/\sqrt{(kt)}$ between $(1\ 5+\gamma)\, b/\sqrt{(kt)}$ and $(1\ 5+2\gamma)\, b/\sqrt{(kt)}$ between $(2\ 5+2\gamma)\, b/\sqrt{(kt)}$ and $(2\ 5+3\gamma)\, b/\sqrt{(kt)}$ etc $]$

Take $\gamma = a/b = \frac{1}{10}$, i e suppose that the outer $\frac{1}{11}$ part of the muscle produces no heat Consider a muscle 1 1 mm thick on each face of the thermopile, i e put $b = 0\ 1$ cm, and take $k = 1\ 35 \times 10^{\ 3}$ Then the curve of fig 4 can be calculated, showing how a rise of temperature of 1 10 produced by the sudden heating of the inside $\frac{10}{11}$ of the muscle drops in a few seconds to 1 00 as the heat is redistributed For a muscle 1 mm thick on each face of the thermopile, 1 2 sec is required for the temperature to fall half way to its final level Thus apparent "delayed negative heat" may readily be observed in a muscle the outside of which is not functioning

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* $\frac{2}{\sqrt{\pi}} \int_0^x e^{-x^2} dx$

With a thick muscle the effect may continue for several seconds It was frequently observed with sartorii of Hungarian frogs, and provided one reason for discarding them The conclusion that this delayed negative heat is not a genuine physiological phenomenon was confirmed by the fact that its time relations, at different temperatures, agreed in general with calculation With the thinner muscles of English frogs thermal equilibration is much quicker the time taken to any stage is proportional to the square of the thickness Thus in fig 4 for a muscle 0 4 mm thick, the times should be divided by (1 1/0 4)^2 = 7 6, so the whole process would be half complete in 0 16 sec , 90 % complete in 0 36 sec It can still sometimes be seen but the muscles of English frogs appear to behave more uniformly and it is seldom troublesome

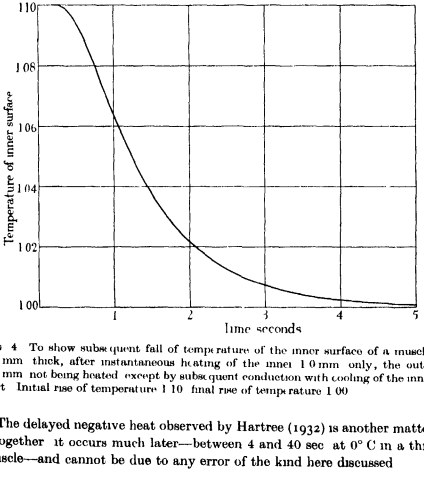

FIG 4 To show subsequent fall of temperature of the inner surface of a muscle, 1 1 mm thick, after instantaneous heating of the inner 1 0 mm only, the outer 0 1 mm not being heated except by subsequent conduction with cooling of the inner part Initial rise of temperature 1 10 final rise of temperature 1 00

The delayed negative heat observed by Hartree (1932) is another matter altogether it occurs much later—between 4 and 40 sec at 0° C in a thin muscle—and cannot be due to any error of the kind here discussed

The effect of non-uniform heating occurs very obviously in one special case, and explains a phenomenon which was, for a time, very hard to understand When a muscle lifts a load isotonically for a limited distance, and then comes against a stop, subsequent lengthening in relaxation is very rapid The tension developed by the muscle, while contracting against the stop, may be considerably greater than the load, and in relaxation the weight begins to drop only when the tension is already falling rapidly The energy of the load, i e the work done in lifting it, then appears suddenly in the muscle as heat (see fig 8 below) and a very sharp upstroke occurs on the photographic record This upstroke often shows two striking anomalies (see p 169 below)

(i) it is greater than can be accounted for by the energy of the weight, and

(ii) it is followed by a rapid fall (in a second or two) to a level which *can* be accounted for by that energy

If these effects were genuine expressions of physiological activity, there would have to be a sudden heat production in relaxation far greater than the energy of the load followed by a large negative heat immediately afterwards, exactly compensating for the excess Suppose, however, that the part of the muscle near the thermopile relaxes rather more slowly than the part outside Then during lengthening the load would be borne chiefly by the more slowly relaxing part of the muscle, which would absorb an undue proportion of its energy, and so non-uniform heating would result The part near the thermopile would be heated more than the part outside and the initial deflexion would be too great it would then diminish to a steady level in a second or so, depending on the thickness of the muscle and on the relative positions of the differently heated parts This is what is observed

The difference required in time of relaxation of the different parts of the muscle would not need to be great The lengthening may begin (at 0° C) nearly 1 sec after the end of the stimulus, and may be complete in 0 1 sec If the inner portion of the muscle relaxed 10 % more slowly than the outer portion, the difference would be sufficient The supply of oxygen to, and the loss of CO_2 from, the inner portion is not so rapid as for the outer portion, which might be the cause of it The fact that, after thermal equilibration, the extra deflexion observed in relaxation is closely accounted for by the energy of the load gives one confidence in this explanation of the anomalies observed

(*g*) *On the possibility of using a resistance thermometer for muscle heat measurements* All successful work hitherto on the heat production of isolated muscle (or nerve) has employed thermocouples or thermopiles

An obvious suggestion is that a resistance thermometer might be used A wire with a large temperature coefficient of resistance is made one arm of a bridge At any given temperature the bridge is balanced with a galvanometer a rise of temperature of the muscle raises the resistance of the wire, throws the bridge out of balance and causes a deflexion of the galvanometer

The sensitivity of a given resistance thermometer is directly proportional to the e m f employed in its bridge Both might be increased indefinitely were it not for the heat liberated in the resistance wire itself In practice, if that heat is too great, disturbances occur which render the increase of sensitivity useless In myothermic experiments such disturbances are particularly obvious, for the muscle (for quickness of recording) must lie in close contact with the heated wire, and differences of temperature are set up between different points in it When the muscle contracts cooler regions come in contact with the wire, and the negative effect of these may entirely mask the positive effect due to the heat production of the muscle itself

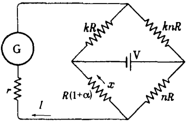

FIG 5 Bridge for resistance thermometer (arm $R(1+\alpha)$), in balance when $\alpha = 0$

The theory of the resistance thermometer applied to myothermic experiments is given below in its ordinary form it is shown to have no advantage in sensitivity over the thermopile, and certain disadvantages This prediction was tested experimentally by constructing a resistance thermometer of 10μ platinum wire and using it with a pair of frog's sartorii under optimum conditions (see below) Its sensitivity was less than that of a thermopile, even when disturbances due to the heat set free in it were so great as to render it useless A resistance thermometer of extremely thin strip or film would be better adapted than a thermopile, particularly with alternating current, to valve amplification The conditions of its employment are discussed It might be badly disturbed by stimulation of the muscle, by "calibration", or even by action potentials

The bridge shown in fig 5 is in balance when α is zero We suppose that the muscle lies in close thermal (but not electrical) contact with the wire of resistance R, and that when it contracts its heat production warms the wire and so increases its resistance to $R(1+\alpha)$ The quantity α is very small, which simplifies the calculation Let V be the battery voltage, r the resistance of the galvanometer, x the current in the wire R, E and I respectively the e m f across and the current through the galvanometer Then, sufficiently nearly,

$$I = \frac{V\alpha}{(1+n)[R(1+k)+r(1+1/n)]}, \tag{1}$$

$$E = \frac{V\alpha}{(1+n)[R(1+k)/r+(1+1/n)]}, \tag{2}$$

$$x = \frac{V}{R(1+n)} \tag{3}$$

Let the maximum allowable rate of heat production in the wire R be Q watts Then

$$Q = Rx^2 = V^2/R(1+n)^2$$

Substituting for $V/(1+n)$ in (1) and (2), we find

$$I = \frac{\alpha\sqrt{(RQ)}}{R(1+k)+r(1+1/n)}, \tag{1'}$$

$$E = \frac{\alpha\sqrt{(RQ)}}{R(1+k)/r+(1+1/n)} \tag{2'}$$

With the limitation that Q must not exceed a certain value, we require to make I, or E, as great as possible

It is clear from (1′) that by making n large and/or k small we can increase I For the greatest conceivable advantage take $k = 0$ and $n = \infty$ Then

$$I = \frac{\alpha\sqrt{(RQ)}}{R+r}.$$

This, if R is variable, can be shown to be greatest when $R = r$, when it becomes

$$I = \tfrac{1}{2}\alpha\sqrt{(Q/r)} \tag{4}$$

Now the current sensitivity of a well-designed galvanometer of given period is approximately proportional to the square root of its resistance, so that the maximum deflexion resulting from a current I in a galvanometer of resistance r is proportional to $I\sqrt{r}$, which from (4) is proportional to $\frac{1}{2}\alpha\sqrt{Q}$ and is therefore independent of r Hence we can take any convenient sensitive galvanometer of given period and the maximum deflexion will be about the same

To find an approximate upper limit to the permissible current in the bridge, let us take Q to be equal to the rate of resting heat production of a frog's sartorius muscle in oxygen at room temperature. This is of the order (calculated from Hill 1928) of 4×10^{-5} W. The resting heat production of a muscle at 20° C, with an ordinary thermopile, gives a steady deflexion of the same order of size as that caused ballistically by a single twitch, and a rate of heat production in the wire greater than this would almost certainly cause disturbances when the muscle contracted.

Let us calculate the current sensitivity per 1° C so that α becomes the temperature coefficient of resistance. The highest value of α given in Kaye and Laby's Tables is 6.2×10^{-3} for iron and for nickel. The value for platinum, a metal which would be more convenient for very fine wires, is 3.8×10^{-3}. With $\alpha = 6.2 \times 10^{-3}$, $Q = 4 \times 10^{-5}$, and $r = 40$, the maximum current through the galvanometer, per 1° C, becomes (from equation (4))

$$I = 3.1 \times 10^{-6} \text{ amp}$$

The thermopile (P 4) chiefly used in the present work has a resistance of 32 ohms and gives about $1400\,\mu\text{V}/1°$ C, which with a galvanometer of 40 ohms provides about 20×10^{-6} amp, about 6 times as much as the resistance thermometer. Another thermopile, with more couples, but not so thin, gives $3640\,\mu\text{V}/1°$ C with a lower resistance. It would be nearly 20 times as sensitive as the resistance thermometer. It might be suggested that the limiting value of Q taken above is rather too low: the sensitivity, however, is proportional only to the square root of Q, so that to equal the sensitivity even of the thin thermopile, the resistance thermometer would require a current producing heat at 36 times the rate assumed above. This would probably cause intolerable disturbances. The thermopile has no errors at all of this kind. In the calculation we have taken throughout the most favourable conditions. We conclude, therefore, that the thermopile has a far higher current sensitivity than the resistance thermometer for myothermic experiments. Incidentally, being differential, it is not affected by gradual changes of temperature: a resistance bridge would require continual readjustment unless designed to be differential.

We have assumed that the resistance thermometer is employed with a galvanometer, in the same way as the thermopile. It is possible that the resistance element might be constructed of exceedingly fine wire or strip, or of metal film sputtered on mica, and so give a higher volt sensitivity suitable for use with amplifier and oscillograph. Employed in this way it would have the advantage that an alternating current could be used in the bridge, which would be easier to amplify than the direct current produced by a thermopile From (2′) above the maximum possible e m f can be calculated as before by putting $\alpha = 3{\cdot}8 \times 10^{-3}$ (for platinum), $Q = 4 \times 10^{-5}$, $k = 0$ and $n = \infty$ r is now the input impedance of the first valve which may be taken as infinite compared with R Hence, for 1° C rise of temperature,

$$E = \alpha\sqrt{(RQ)} = 24 \times 10^{-6}\sqrt{R}$$

The volt sensitivity, therefore, increases as the square root of the resistance of the element, so the latter resistance should be as great as possible The specific resistance of platinum is about 11×10^{-6} A wire therefore, 4 cm long and $10\,\mu$ in diameter has a resistance of 154 ohms, giving (from above) about $300\,\mu$V/° C With Wollaston wire of $2\,\mu$ diameter the resistance would be 25 times and the volt sensitivity 5 times as great, say $1500\,\mu$V/° C If it were possible to construct a film of sputtered platinum on mica, of resistance 100,000 ohms, the volt sensitivity would be about $4000\,\mu$V/° C This is nearly three times as great as that of the best thermopile made with very fine rolled wire, but about equal to that of thicker thermopiles with more couples

If, therefore, such resistance elements could be constructed they would have a high volt sensitivity, and the advantage that they could be used with alternating current They would certainly have a very small heat capacity, and so would allow very rapid recording It is probable, however, that far more serious errors might result from leakage of the stimulus than with a low resistance circuit The insulation (for quickness) must certainly be thin, and even if its resistance were infinite it would allow a capacitative leak between the muscle and the resistance element A wet muscle separated by only (say) 10–$20\,\mu$ of insulation (as in the present thermopiles) from the conducting system is subjected to stimuli of several volts, and—if control heating is needed—to a condenser discharge of several hundred volts An insulation resistance of 20 megohms (and more could not be expected after soaking in Ringer s solution) might allow only 1 part in 200 of an e m f applied to the muscle, to appear between the ends of a 100,000 ohms strip With a 6 V stimulus however, this would be 60 mV, thousands of times greater than the quantity to be observed, and a considerable part of it would be amplified with "control heating" the disturbance would be still greater Even with a low resistance element, if its insulation is not very good a discharge between the ends of the muscle may be slightly short-circuited through insulation and galvanometer, and appreciable disturbance may occur For that reason it has always been evident that the insulation of a thermopile must be kept high With a high resistance circuit, however, and amplification, leakage of stimulating (and *a fortiori* of heating) current could scarcely fail to disturb the recording system

It might be suggested that the muscle could be stimulated through its nerve, and not directly With indirect stimulation, however, of the frog's sartorius, or of any similar muscle owing to the difficulty of preparing the nerve without injury, there could be no certainty that *the whole* of the muscle would respond equally For an accurate study of the time relations of the heat production it is essential that the whole muscle should contract together otherwise heat flow from one part to another may mask the true effects Even, however, if indirect stimulation were permissible, the action potential of the muscle itself might leak, either directly, or through the very high capacitance of the muscle separated from the element by only 10 μ of mica, into the recording system Action potentials of 20 mV, separated by only 10 μ of wet mica from a high resistance connected to an amplifier, might of themselves alone produce disturbances greater than the 4–8 μV which is the whole quantity to be measured It might be possible to design an amplifier to avoid the effect of such leaks, but its design would be complex, and with the very large disturbances to be expected, particularly in direct stimulation no certainty of success could be predicted

We may conclude therefore, that for measurement of muscle heat

(*a*) a resistance element, when used with a galvanometer, is far inferior to a thermopile,

(*b*) a resistance element to be used with alternating current and valve amplification must have a very high resistance,

(*c*) with an element of high resistance, leaks and disturbances from stimuli, and even from action currents, might prove prohibitive,

(*d*) and therefore that the use of a resistance thermometer, instead of a thermopile, would probably require years of experimental development, with no security of ultimate success

It seems better to carry on with the efficient thermopiles and galvanometers already available

PART II THE HEAT OF SHORTENING AND LENGTHENING AND THE INFLUENCE OF LOAD ON THE RATE OF ENERGY LIBERATION

(*a*) *The extra heat associated with shortening* When a muscle mounted on a thermopile, in the manner and with the precautions described above, is tetanized isometrically, the galvanometer record of its heat production is similar to curves A and E, fig 6, and curves A, E and K, fig 7 If it is allowed to shorten isotonically under a small load, the record rises initially more rapidly, as in curves B, C and D fig 6, and then becomes approximately parallel to the isometric curve The extra deflexion is proportional to the amount of shortening allowed

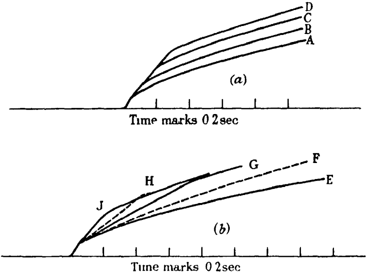

FIG 6 Heat production during isotonic shortening from the start Curves traced from galvanometer records and superimposed Tetanus at 0° C muscle 32¼ mm long, 88 mg time, 0 2 sec (a) *Upper* Shortening different distances under constant load 1 9 g A, isometric, B, 3 4 mm, C, 6 5 mm, D 9 6 mm (b) *Lower* Shortening constant distance 6 5 mm under different loads E, isometric, F, 31 9 g, G, 23 7 g, H, 12 8 g, J, 1 9 g

If a muscle is stimulated isometrically and then suddenly released under a small load, it shortens rapidly and during its shortening the galvanometer gives a quick extra deflexion In fig 7 (c), K represents the heat deflexion for a contraction which is isometric throughout L for a contraction which is isometric at first, with release at 1 8 sec followed by 9 1 mm shortening under 2 9 g load M for a similar release at 0 95 sec N for the same release at the start The extra deflexion is the same whenever the release occurs, provided that the contraction is fully maintained It is proportional to the amount of shortening allowed, as is seen from fig 7 (a), curves A to D

This sudden extra deflexion of the record implies a sudden increment in the rate of heat production of the muscle The increase of heat rate is proportional to the speed of shortening and stops when the shortening of the contractile component stops the total extra heat is proportional to the total shortening If the load is small, the speed of shortening is high and the increment in the heat rate is large if the load is greater, the speed of shortening is less and the increment in the heat rate is smaller In fig 6 (*b*), curve *E* represents the same isometric contraction (45 g tension) as *A* *J*, *H*, *G* and *F* represent isotonic contractions of 6 5 mm, released at the start, under 1 9, 12 8, 23 7 and 31 9 g respectively With the greatest load the shortening was not complete in the time The heat rate was greater the less the load and the more rapid the shortening but the total extra

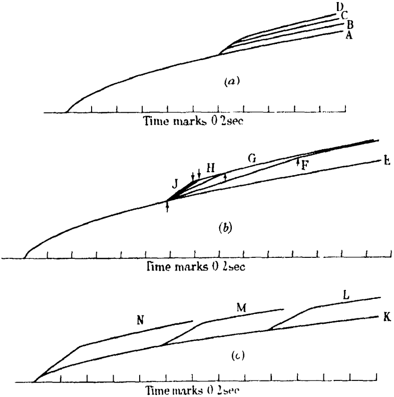

FIG 7 Heat production in isotonic shortening released during isometric contraction Curves traced from galvanometer records and superimposed Tetanus at 0° C muscle, (*a*) and (*b*) 29 5 mm, 89 mg (*c*) 30 mm 87 mg time 0 2 sec (*a*) *Upper* Release at 1 2 sec shortening different distances under constant load 3 0 g *A*, isometric, *B*, 1 9 mm, *C*, 3 6 mm, *D*, 5 2 mm (*b*) *Middle* Release at 1 2 sec, shortening constant distance 5 2 mm under different loads *E*, isometric (45 g) *F*, 24 9 g, *G*, 13 9 g, *H*, 5 7 g *J*, 3 0 g End of shortening shown by arrows (*c*) *Lower* Isometric (*K*), release at 1 8 sec (*L*) 0 95 sec (*M*), and start (*N*), shortening constant distance 9 1 mm against constant load 2 9 g

heat was the same in each case In fig 7 (*b*) curve *E* represents the same isometric contraction (43 g tension) as *A* *J*, *H*, *G* and *F* represent isotonic contractions of 5 2 mm, released at 1 2 sec, under 3 0, 5 7, 13 9 and 24 9 g respectively Again the extra heat rate is less with the greater load, but the total amount of heat is the same

With a greater load, proportionally more work is done by a muscle in shortening more slowly a given distance, but *the total heat for shortening remains unaltered* Thus when a muscle shortens it liberates extra energy in two independent forms, (i) heat for shortening proportional to the shortening, and (ii) mechanical work

The evidence for these conclusions is illustrated in figs 6 and 7 The curves were traced from actual records and superimposed Details of the calculation required for an accurate determination of the heat of shortening are given below (p 171)

The heat for shortening 1 cm is very constant from one muscle to another, when due account is taken of the size of the muscle In fourteen experiments at 0° C its average variation from a mean value was only 13 %, which includes all experimental errors (see Table III below) Let a muscle shorten by an amount x cm, and let the shortening heat (which is proportional to x), expressed in energy units, be ax g cm Since x has the dimensions of length and ax the dimensions of work a must have the dimensions of force and we reckon it in grams weight The shortening heat can be expressed *as though* it were derived from the work done in shortening a distance x cm against a frictional resistance of a g wt

The quantity a clearly depends on the size, in particular on the cross-section, of the muscle In a muscle of given length but of n times the cross sectional area the heat for shortening must be n times as great In a muscle of mass M, of density ρ, of length l and of uniform cross-section A, $A = M/\rho l$ the heat for x cm shortening is ax g cm, so the apparent resistance to shortening, from which the heat can be calculated, is a/A g wt/sq cm of cross-section At 0° C in the frog's sartorius the mean value of a/A in a maximal contraction is about 400 g wt/sq cm

The value of a should clearly depend on the strength of contraction if, for example, only half the fibres of a muscle contracted, a would be reduced to one-half The simplest measure of the strength of contraction is P_0, the full isometric tension We should expect a/P_0 to be constant In eleven experiments the mean value of a/P_0 was about 0 25, and the average variation from the mean was only 10 % a is not very easy to measure and a considerable part of this 10 % must be experimental error Thus the apparent resistance to shortening bears a rather constant ratio to the maximum force which a muscle can exert

The ratio a/P_0 is constant, or nearly constant, not only from one muscle to another, but at different temperatures At a higher temperature a is greater, but P_0 is greater too Thus a/P_0 may be regarded, at least as a first approximation, as a natural constant for frog's muscle If a frog's sartorius can exert a maximum force P_0, then in shortening a distance x it will liberate heat approximately equal to $P_0x/4$ g cm , which is independent (i) of the speed of shortening, (ii) of the work done, and (iii) of the temperature

(*b*) *The rate of energy liberation during shortening* We have seen that in shortening a distance x cm , extra heat ax g cm is set free If P g be the load lifted, the work done is Px g cm Thus the total energy, in excess of isometric, is $(P+a)\,x$ g cm The *rate* of extra energy liberation, therefore, is $(P+a)\,dx/dt$, or $(P+a)\,v$, if v be the velocity (cm /sec) of shortening

It is found experimentally that the rate of extra energy liberation, $(P+a)\,v$, is a rather exact linear function of the load P, increasing as P diminishes, being zero when $P=P_0$ in an isometric contraction and having its greatest value for zero load (see fig 11 below) We may write therefore

$$(P+a)\,v = b(P_0-P), \tag{1}$$

where b is a constant defining the absolute rate of energy liberation This may be written

$$(P+a)\,(v+b) = (P_0+a)\,b = \text{const} \tag{2}$$

Equation (2) relates speed and load in an isotonic shortening, it can be verified separately without any thermal measurements this will be referred to later (p 177) It represents a rectangular hyperbola with asymptotes at $P=-a$, $v=-b$

The constant b is of the dimensions of a velocity (cm /sec) and, when due account is taken of the dimensions of the muscle, is very constant at a given temperature If a muscle of given cross-section be n times as long, then v, the velocity of shortening, will obviously be n times as great under a given load Hence b also should be proportional to the length l of the muscle At 0° C in the frog's sartorius the mean value of b/l was about 0 33/sec (Table III below), and the average variation from the mean in eleven experiments was only 9 % Of this variation a considerable part must have been due to experimental error, so that the real variation is less Thus b/l is constant, or nearly constant, from one muscle to another at a given temperature

It is well known that muscles can shorten, and give out energy, much more quickly at a higher temperature It was expected therefore that b

would increase with temperature This was verified experimentally and b was found to be increased about 2 05 times for a rise of 10° C

(*c*) *The energy absorbed during lengthening* In the experiments illustrated in fig 7 during an isometric contraction a muscle was released and allowed to shorten and do work The converse experiment has been made (fig 13 below) a muscle was allowed to contract isotonically through a limited distance, and then a load somewhat greater than the isometric tension was applied to it The muscle lengthened and work was done upon it by the load The questions to be answered were

(i) Is there a negative heat of lengthening, the converse of the positive heat of shortening?

(ii) Is the work done by the load in falling simply transformed into heat, or is it absorbed, or dealt with in some other way?

In principle, equation (2) above, viz $(P+a)(v+b) = (P_0+a)b$, could apply just as well to lengthening as to shortening For shortening v is positive and P is less than P_0, as we know In isometric contraction v is zero and $P = P_0$ In lengthening v is negative and P should be greater than P_0 Now the fact that the force exerted during lengthening is larger than in isometric contraction, and is greater the more rapid the lengthening, has been known since Fick (1882), and was examined in detail by Wyman (1926) and by Levin and Wyman (1927) This qualitative confirmation that equation (2) applies to lengthening as well as to shortening encouraged one to proceed

The difficulty in making thermal experiments on lengthening is that, unless the speed of lengthening is very low, the force developed will be too great for the muscle to bear For $v = -b$, i e for a speed of lengthening only about one-quarter of the maximum speed of shortening, equation (2) shows that P should be infinite Even for $v = -b/6$ (in an ordinary frog's sartorius, about 1 6 mm /sec) P should be 1 25 P_0 The reason why high tensions must be avoided is that active muscle apparently "gives", or ' relaxes", or "slips", rather like a wire strained beyond its elastic limit, if the force is too great (see Gasser and Hill 1924, p 412) No damage is necessarily done to the muscle, and the active state is set up again as soon as the force is reduced For the moment, however, a rapid "give" or "slip" occurs when the load is too great, and the work done by the load is degraded directly into heat, as it is in ordinary relaxation when the stimulus ends (see fig 8 below)

It is necessary, therefore, to work with loads not very much greater than the isometric tension, and such loads are bound to stretch the muscle rather slowly The extra heat for lengthening x cm should be $-ax$ g cm but x must be small if the stretch is to be slow and the contraction not unduly prolonged the heat therefore is bound to be small, and to appear only slowly Moreover, it is apt to be masked by heat produced irreversibly through "give" or "slip" of parts of the muscle With the additional complication that the isometric heat rate varies somewhat with length, it has proved impossible as yet to give a decisive answer to the question—Is the heat of lengthening negative and equal to $-ax$ g cm ? The evidence (see below, p 179 and figs 13 and 14) inclines to the view that it *is* negative, but the experimental determination of its absolute amount has proved, as yet, to be too difficult Perhaps another muscle (e g of a tortoise, cf Wyman (1926), or a dog-fish, cf Levin and Wyman (1927)), or a frog's muscle at another season, might provide less difficulty

The second question—Is the work done in stretching the muscle transformed into heat, or is it dealt with in some other way?—can be answered decisively (see below, p 180 and fig 14) It is not transformed into heat unless the load is too great and "give" or slip" occurs With a suitable load, e g of 60 g on a muscle capable of an isometric tension of 50 g, giving a rate of stretch of about 1 4 mm /sec, the work done may disappear, almost or entirely, with little or no remainder as heat With greater loads, however, and quicker stretches, a large fraction of the work done by the load in falling is transformed into heat and causes a rapid upstroke of the galvanometer

It may be asked—What happens to this work? The answer might be that we must think of some mechanism like that of a tramway motor, capable of acting as a dynamo and feeding back current to the mains in running down hill The true answer, however, as we shall see below, may well be that the work *is* transformed into heat, but the rise of tension associated with stretching diminishes the rate of energy liberation of the muscle so much that the positive heat associated with the degradation of work is balanced by the diminished heat production of the muscle It is not necessary to suppose that any part of the mechanism is really reversed only that it is slowed

(*d*) *The nature of the heat of shortening and the mechanism of the energy control* The exactness of the relations described above invites the question of their nature and mechanism Is the heat of shortening simply degraded work, done in overcoming a constant resistance a? if so, it should occur also as positive heat in lengthening, since processes which degrade work into heat act equally in both directions The question cannot be answered decisively until that of the heat in lengthening has been finally settled If the heat of shortening were degraded work, if a were a constant internal resistance, we might expect it, as viscosity is, to be largely reduced by a rise of temperature actually it is increased somewhat, in proportion to the strength of contraction It is difficult to think of a force of this kind which might be present in muscle

An objection to the idea that *a* might represent a real, irreversible frictional resistance to shortening, or lengthening, is that, if it did, there should be a difference $2a$ between the loads (i) at which a muscle just shortens, and (ii) at which it just lengthens $2a$ is a fairly large quantity, about half the isometric tension the difference in question, if it exists, is certainly much smaller than this If, however, the heat of shortening be due to a ‘ reversible” change, in the sense at least that its sign is reversed in lengthening, and if *a* be only an apparent resistance, then we must think of some physical process which gives out heat in shortening, absorbs heat in lengthening Consider, for example, the case of surface tension This has a negative temperature coefficient, so that heat is given out when an area exhibiting surface tension contracts and does work, absorbed when the area is made to expand The surface tension, for example, of benzene at T° K is given as $\sigma_T = \sigma_0 - 0\ 146(T - T_0)$ The heat given out, therefore, in an air-benzene surface is $T \times 0\ 146$ ergs/sq cm of contraction, which at 0° C is 40 ergs or about 0 04 g cm Or we might speculate about the heat given out when chemical affinities are satisfied by the folding up of myosin chains during muscular shortening (cf Astbury 1938), and the heat absorbed when the molecules are drawn apart again Without, however, a more concrete picture of the actual muscular mechanism, it is not very useful to speculate further It is sufficient to realize that plausible theories can be invented to imitate this property of muscle

The control exercised by the tension *P* existing in the muscle at any moment, on the rate of its energy expenditure at that moment, may be due to some such mechanism as the following Imagine that the chemical transformations associated with the state of activity in muscle occur by combination at, or by the catalytic effect of, or perhaps by passage through, certain active points in the molecular machinery, the number of which is determined by the tension existing in the muscle at the moment We can imagine that when the force in the muscle is high the affinities of more of these points are being satisfied by the attractions they exert on one another, and that fewer of them are available to take part in chemical transformation When the tension is low the affinities of less of these points are being satisfied by mutual attraction, and more of them are exposed to chemical reaction The rate at which chemical transformation would occur, and therefore at which energy would be liberated, would be directly proportional to the number of exposed affinities or catalytic groups, and so would be a linear function of the force exerted by the muscle, increasing as the force diminished

The isometric contraction, on this view, does not present a special case Let the rate of total energy expenditure by a muscle exerting a force P_1 be H_1, and exerting a force P_2 be H_2 then the experiments referred to above have shown that

$$H_1 - H_2 = b(P_2 - P_1)$$

If H_1 is greater than H_2 it is because, on this view, more chemically reactive points are exposed when the tension of the muscle is less In an isometric contraction the rate of heat production is H_0, corresponding to tension P_0 If the load is lowered to P_1 the muscle shortens and does work, more energy is required, and more energy is actually liberated through an increase, proportional to $(P_0 - P_1)$ in the number of chemically reactive points If the load is raised to P_2 the muscle lengthens and does negative work (work is done on it), less energy is required, and less energy is actually liberated since the number of chemically active points is diminished proportionally to $(P_2 - P_0)$

The question, therefore—Where does the work done on the muscle in lengthening go to?—is answered By raising the tension one causes a decrease in the rate of chemical transformation in the muscle It is not necessary to imagine any actual reversal in the direction of chemical reaction during lengthening only a slowing of the reaction normally accompanying activity It has never been found, even with more rapid stretches, that the total energy rate of the muscle is negative it is less than the isometric energy rate but still positive Lengthening, therefore, and negative work, do not cause a reversal, but only a slowing, of the processes associated with activity

The high temperature coefficient of the constant b is presumably the resultant effect of the temperature coefficients of the chemical reactions by which the energy is liberated For a given tension in the muscle the number of chemically reactive regions may be the same at different temperatures if so, the temperature coefficient of b is simply that of the chemical processes Possibly, however, for a given tension, the number of reactive spots may alter somewhat with temperature if so, the temperature coefficient of b is a mixture of both effects As defined above, b is a linear velocity (cm /sec) but if $n(P_2 - P_1)$ be the actual increase in the number of reactive spots, in passing from a higher tension P_2 to a lower tension P_1, then b/n is a rate of energy liberation We have no idea what

n may be and it is simpler to leave matters as they are, remembering that b is expressed in cm /sec simply because, for convenience, the number of active spots is taken as equal, and not merely proportional, to (P_2-P_1)

The isometric heat rate at 0° C, between 0 8 and 1 8 sec from the beginning of stimulation, when the tension has become steady, lies consistently between 2 and 4×10^{-3}, with a mean of about 3×10^{-3} cal /g × sec = 128 g cm /g × sec The extra heat rate associated with shortening is greatest when $P=0$, when it is of the order of 500 g cm /g × sec Thus during the limited interval of shortening under zero load the total heat rate may be 5 times as great as during an isometric contraction The effect, therefore, of rapid shortening on the heat production is large

During lengthening the work done *by* the muscle is negative, so the work done *on* the muscle must be subtracted from the observed heat to give the energy actually liberated by the muscle The net energy rate of the muscle, so calculated, would become zero if $-Pv$, the rate of doing work on the muscle, became equal to the isometric heat rate, which—taking its mean value at 0° C—is 128 g cm /g × sec With the usual values for 0° C of a, a/P_0 and b, it can be calculated that $Pv=-128$ g cm /g × sec when $P/P_0=1$ 30 and $v=-0$ $2b$ These represent a load 30 % greater than the isometric tension, a speed of lengthening in an ordinary frog's sartorius of about 2 mm /sec This may explain why a discontinuity occurs, and the muscle "gives" or "relaxes", with a load not very much greater than isometric The total energy rate by the muscle gets reduced too nearly to zero, and the contraction cannot be maintained In terms of our hypothesis, an increased tension on the muscle may obliterate all the chemically reactive points of the mechanism, chemical transformation may become impossible, energy liberation may cease, and the muscle may relax as though the stimulus had been stopped Mechanically this is what happens, as is easy to verify by suddenly subjecting a fully contracting muscle to a load (say) 50 % greater than the isometric tension and it is interesting that it should happen in the very region of loads where calculation would predict it

At a higher temperature the isometric heat rate is greater, but b is greater too "Reversible" lengthening, therefore, can be quicker, but the relative load, P/P_0, at which the discontinuity occurs should be about the same

(e) *Shortening heat and relaxation heat during an isometric contraction* In the heat records of isometric contractions in figs 6, 7, 8, 13 and 14, the upstroke at the beginning is very similar to that in records of contractions in which shortening is permitted but smaller This, as will be shown below, is almost certainly due to shortening of a contractile component of the muscle, stretching (*a*) an elastic component of the muscle, (*b*) its tendons, (*c*) the connexions to the isometric lever, and (*d*) the lever itself. The quick upstroke at the beginning represents ‘shortening heat”. The work done in stretching these several elastic bodies remains as mechanical potential energy until relaxation occurs, when it appears as heat. It is the origin of the obvious “hump” on the heat record which occurs in relaxation (see fig. 8, curve *A*, below). This ‘hump’ is not the only source of heat during relaxation but is a considerable part of it. It can be reduced by fixing the muscle rigidly at its ends, so that less work is done against elastic forces, but it is doubtful whether it can be altogether abolished. The muscle may have within itself a genuine undamped elastic component: its machinery may be elastic. (See p. 187 below.)

The heat rate during isometric contraction and relaxation is largely affected by these mechanical factors. The present instruments are capable of providing a much more accurate analysis of the initial heat than any hitherto published. It is hoped to complete such an analysis in the near future, with adequate control of the mechanical conditions affecting the heat.

(*f*) *The sudden fall in the heat rate at the end of stimulation.* With the present instruments the moment at which a stimulus stops can easily be detected on the heat records by an ‘angle’, i.e. by a sudden change of slope. It is clear that the heat rate immediately begins to drop as soon as stimulation ends. An analysis of the records makes the drop very obvious, and simultaneous isometric mechanical records show that the tension is fully maintained for some tenths of a second (at 0° C) afterwards.

In a twitch provoked by a single shock the heat has a high rate at first, declining gradually to zero. The visible mechanical effect, however, only gradually reaches its maximum. It is natural, therefore, that in a maintained tetanus the full tension should remain for some moments after the heat rate has begun to fall. The earlier events in the chain of chemical processes accompanying muscular activity, although producing heat, may not necessarily be those which are responsible for the maintenance of tension.

(*g*) *The heat of relaxation after an isotonic contraction.* When a muscle contracts and relaxes isotonically the work done on the load during shortening appears in the muscle as heat during relaxation. If the load be great the work is considerable and the deflexion when relaxation occurs is sudden and large. This deflexion is quantitatively equivalent to the work.

In fig. 8, *B* is the heat record of the isotonic contraction of a muscle lifting a large load, and shortening slowly as shown by the mechanical record *b* below As soon as the shortening was complete the large after-load was held up, by the device described above (p 145), and the muscle relaxed very late, when its tension had fallen to a low value equal to the small initial load The heat record *B* showed only a very small "hump" corresponding to the degradation to heat of the potential energy of its elastic component as the tension of the muscle relaxed

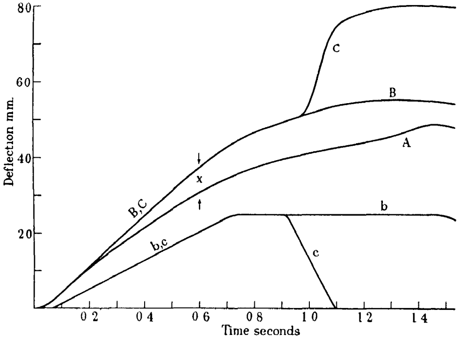

FIG 8 Relaxation heat in isometric and isotonic contractions *A*, *B* and *C*, galvanometer records of heat in 0 6 sec tetanus at 0 C with initial load 3 6 g Muscle 196 mg *A*, isometric, *B*, isotonic after load 38 6 g, held up, *C*, isotonic, same after load but free *B* and *C* rise faster than *A*, owing to the shortening heat When the muscle lengthens under the load, the energy of the load (the work done by the muscle on it) appears as heat hence the rapid upstroke in *C* during relaxation *C* rises above *B* by an amount equal to the work Efficiency, 30 5 % Mechanical records below, with corresponding letters Relaxation under the small initial load only (*b*) was very delayed Note the small relaxation "hump" on the heat record *A* of the isometric contraction All records are means of a pair in a series and reverse Y, end of stimulus

When the load was free to fall as the muscle relaxed, the mechanical record was *c* and the heat record *C* The rise of the heat record coincides in time with the fall of the load, and the maximum height of *C* above *B* represents a production of heat equal to the work of the load in falling This equality is not always apparent Sometimes the sudden rise of the heat record is greater than the work, but in such cases it is followed by a rather rapid fall apparently representing an absorption of heat The time to complete this subsequent fall is of the same order of size as that calculated (see p 152 above) for the redistribution of heat unevenly produced in the muscle When the difference between B and C has become steady it corresponds to the work Relaxation is very sudden with large loads, and if the inner layer of muscle, that nearer the thermopile, relaxed slightly later than the outer layer, it would be stretched by the falling load and would receive more than its fair share of the energy of the latter this would then be redistributed by conduction, but only after it had affected the thermopile The anomaly has no physiological significance, it is an excellent example, however, of the traps which beset myothermic experiments Without a critical examination it might have led to very peculiar conclusions about heat production and absorption during relaxation The facts are really quite simple the energy of the load, and the energy of any stretched bodies in series with the muscle, are turned straight into heat when the muscle relaxes

In fig 8, curve A represents an isometric contraction it is lower than B for the isotonic contraction owing to shortening heat in the latter Relaxation is later in the isometric contraction The small 'hump" to the right represents elastic energy which is turned into heat when relaxation occurs

The stimulus ended at X, and a small change in the slope of the isometric heat record can just be detected there, representing a fall in the rate of heat production The ' angle" is less obvious than it often is

The mechanical efficiency, i e (work)/(total energy), was high in the experiment of fig 8, viz 30 5 % This was due to the fact that the load was small enough for the muscle to lift it a considerable distance, and yet large enough for the muscle to have to go on lifting it till after the stimulus ended Efficiencies up to 35 % have been observed These efficiencies, of course, cover only the initial process if the recovery process were included they would be about half as great Frog's muscle seems to be rather inefficient as compared with human muscle It is interesting that higher efficiencies have been obtained with isotonic contractions than with the Levin-Wyman apparatus see Hartree and Hill (1928*a*, p 243, etc)

(*h*) *The effect of length on the isometric heat rate* From the heat records of "isometric long" and "isometric short" contractions (figs 6, 7, 8, 13, 14) and from the differences plotted in figs 9 and 10, it is seen that the heat rate is greater at the shorter length In figs 9 and 10, curves E, the change of isometric heat rate is plotted as a function of the distance the muscle had shortened Many series of this kind have been made, and they consistently show that the isometric heat rate becomes greater as the length becomes less, reaches a maximum, and then becomes less again at still smaller lengths The relation has been examined only incidentally, in order to provide the necessary correction in the calculation of the shortening heat it has been studied in detail by Brown and Katz, in relation to the tension of the muscle they will report on it elsewhere

The effect is not due at all to the heat of shortening, for shortening was complete long before the isometric heat rate was measured The present experiments throw no light on its nature It is certainly genuine, and directly contradicts a number of earlier observations by various authors the difference is presumably due to the errors which the present methods, and particularly the protected thermopile, were designed to avoid Blix (1902), for example, concluded that muscle heat increased and diminished with the length of the fibres, and was a surface—not a volume—effect Hill (1913) found in a tetanus that the heat was less the more a muscle was allowed to shorten, and Evans and Hill (1914) and Hill (1925) concluded that this was true for all durations of stimulus

All these results were obtained by reading the maximum deflexion of a galvanometer, none of them by measuring the slope of a continuous record of the heat The existence of the shortening heat was not known, and any mechanical work was added automatically to the total heat when the muscle relaxed A protected thermopile was not used, though an attempt was made by Hill (1913) to avoid some of the errors which it avoids by the use of a ' conical" thermopile Considerable temperature differences may have existed between different points of the muscle The results anyhow seem to be wrong, and the moral may be drawn that all earlier experiments on the heat production of muscles allowed to shorten are suspect and should now be repeated

Fenn and Latchford (1933) found a slight increase in the difference (heat for 0 4 sec tetanus – heat for 0 2 tetanus) with decrease of length Fischer (1935) determined the heat for maintenance of tension by subtracting the heat for a tetanus just long enough for maximum tension to be developed from the heat for a much longer tetanus He found a rise in the heat for maintenance as the length was diminished and then a fall Hill (1925) had obtained similar results The effect depends to some degree on the definition of the "resting length"

Experimental evidence

(i) *The heat of shortening* The evidence for the heat of shortening is various some of it was illustrated in figs 6 and 7 The determination of its absolute amount has been made in three ways (*a*) by a complete numerical analysis of the rate of heat production, before, during and after shortening,

(*b*) by calculating the increment in the rate of heat production, when shortening is permitted, from the increased slope of the heat record, and comparing this with the rate of shortening,

(*c*) by measuring the total upward displacement of a heat record, as the result of a given total shortening

All three methods are complicated by the fact that the extra heat for shortening is measured as an excess above the isometric level and the isometric heat rate is somewhat different at different lengths For accuracy it is necessary to make some allowance for this Since the isometric heat rate varies rather rapidly at first when stimulation begins, and then becomes more constant, the allowance is more accurate for later releases than for shortening at the start

All three methods have led to the same conclusions (*a*) is laborious, and with the quickness of the present instruments it has no significant advantage over the others (*b*) is easily applied and is quite satisfactory the allowance, however, for varying heat rate with length is not so accurate as in (*c*) (*c*) seems to give the best and most consistent results and was adopted in all the later experiments Details of its applications are given in figs 9 and 10 and their legends

The muscle is stimulated isometrically and then released after its full tension is developed It shortens the required amount and becomes isometric again It might be supposed that if the instruments had no lag the reading at the moment when visible shortening ended would give the heat of shortening This is not so When a muscle is released part of the shortening observed is that of the elastic component previously stretched by the contractile component in the preliminary isometric phase, when the muscle again becomes isometric at the shorter length, its contractile component goes on shortening, and stretching the elastic component, until the full tension is developed Thus when shortening is checked the isotonic heat record continues at first to rise faster than the isometric, but at a rapidly decreasing rate This necessitates the extrapolation described in the legend of fig 9

After this extrapolation, it remains to allow for the mean extra heat rate (isometric), over the range of shortening considered The lighter the load, the less will be the time taken in shortening and the smaller the correction required The observed excess heat rates are plotted and a mean calculated for the range of lengths involved this is multiplied by the observed time of shortening, and the product subtracted from the extra deflexion at the extrapolated end of shortening No correction is applied for the time occupied in subsequent "isometric shortening" the correction must be

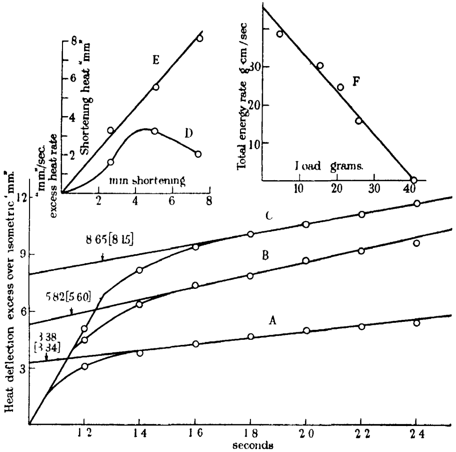

FIG 9 To illustrate the calculation of the heat of shortening *A*, *B* and *C*, heat deflexions (mm) in excess of isometric for 2 65, 5 05 and 7 40 mm shortening under 4 6 g load, after quick release at 1 0 sec Curves extrapolated back to end of shortening, shown by arrows extrapolated deflexion in mm Slow rise after end of shortening due partly to lag of instruments, but chiefly to continued shortening of contractile component against elastic component Curve *D*, excess heat rate (above isometric), when steady, as function of length From this, the mean excess heat rate over the range of shortening was calculated for *A*, *B* and *C*, and a correction applied to the extrapolated deflexion at the end of shortening The result is shown in square brackets, e g [8 15], and plotted in *E* against the amount of shortening ***Result*** $a = 10\ 7$ g wt $= 383$ g/sq cm, $a/P_0 = 0\ 24$ In *F* the total energy rate in another series is plotted against the load result $b = 1\ 12$ cm/sec $= 0\ 325$ length/sec ***Experimental details*** 0° C, thermopile P 5 muscle 101 mg, 34½ mm, mean of series and reverse 1 mm heat deflexion $= 0\ 936$ g cm $= 0\ 218 \times 10^{-3}$ cal/g Average heat rate, isometric, 1–2 sec, 12 2 mm deflexion/sec

small and would be difficult to make The result gives the corrected heat of shortening Plotting this in g cm against cm of shortening a straight line is obtained, the slope of which is *a* This proportionality of shortening heat to distance shortened has been obtained many times the evidence is all similar to that of curves *F* in figs 9 and 10

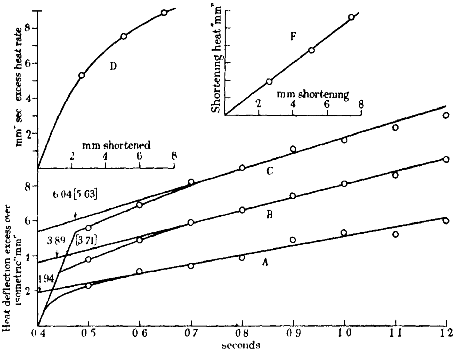

FIG 10 To illustrate the calculation of the heat of shortening Same muscle as for fig 9 but at 13° C and initial load 7 3 g See legend of fig 9 *Result* $a = 14\ 3$ g wt $= 513$ g /sq cm , $a/P_0 = 0\ 193$ *Experimental details* 1 mm heat deflexion $= 1\ 895$ g cm $= 0\ 442 \times 10^{-3}$ cal /g Average heat rate, isometric, 0 4–0 9 sec , 36 6 mm deflexion/sec

Table III contains all the valid results obtained, with weighting factors allotted according to the nature of the experiment Considering the smallness of the quantities observed the results are rather consistent, and the mean values of the ' dynamic constants" there given would seem to be reasonably certain for the case of the frog's sartorius It will be interesting to determine the same constants in other muscles Most muscles are unsuited to thermal experiments of this kind, but for them the indirect method is available, involving only the measurement of the speed of shortening at a series of different loads (see p 177 below)

TABLE III THE DYNAMIC CONSTANTS OF ENGLISH FROG'S SARTORIUS MUSCLE

a is given in g wt /sq cm of muscle cross section
a/P_0 is a number without dimensions
b is given in (muscle length)/sec
The numbers in brackets are the ' weights ' used in calculating the means
For the determination of a, the load lifted was usually small

(i) 0° C

Exp no	13	14	15	16	20	22	23	
a	330 (1)	344 (1)	255 (1)	362 (2)	420 (1)	453 (1)	550 (1)	
a/P_0	—	—	—	0 21 (2)	0 24 (1)	0 28 (1)	0 23 (1)	
b	—	—	—	0 38 (2)	0 34 (1)	0 36 (1)	0 32 (1)	
	24	**26**	**27**	**31**	**33**	**34**	**39**	**Mean**
a	493 (1)	326 (1)	380 (1)	418 (3)	470 (3)	365 (3)	383 (3)	**399**
a/P_0	0 27 (1)	0 31 (1)	0 31 (1)	0 27 (3)	0 28 (3)	0 24 (3)	0 24 (3)	**0 257**
b	0 34 (1)	0 41 (1)	0 38 (1)	0 28 (3)	0 335 (3)	0 30 (3)	0 325 (3)	**0 331**

(ii) Other temperatures

Exp no	36	37	38	39	Mean
Temp 0° C	8 9	13 5	19 8	13	
a	590	613	415	513	
a/P_0	0 28 (3)	0 29 (3)	0 18 (3)	0 19 (3)	**0 235**
b	0 735	0 935	1 09	0 815	
Temp coeff * of b	2 45 (2)	2 16 (3)	1 82 (4)	2 00 (3)	**2 05**

* For 10°, assuming the mean value 0 331 at 0 C and an exponential increase with temperature

(ii) *The effect of load on the shortening heat* In a number of experiments the extra heat for shortening was determined with several different loads, i e at several speeds of shortening, and with very different amounts of work done Release was either at the start, or later, and the methods of calculating the shortening heat were various The shortening heat was always about the same, whatever the load, the speed or the work In three of the latest experiments the method used was that described in detail in the preceding section, which is probably the best, and five series gave the results in Table IV They are summarized at the end of the Table With the smallest loads the shortening heat seems to be rather less than with higher loads with loads more than 25 % of the isometric tension it is practically constant The difference is scarcely significant and may be due to some consistent error The experiments and calculations for the slower shortenings cannot be very exact since the shortening heat is rather small, and when it is spread over a longer time more allowance has to be made for the change of heat rate with length, and the allowance is rather precarious It may be that the difference with the lighter loads is genuine, but in any case it is very small, when compared with the great difference in respect of mechanical work

TABLE IV EXTRA HEAT FOR SHORTENING WITH DIFFERENT LOADS

All heats are given in mm galvanometer deflexion

Exp 33 Temp 0° C Isometric tension $P_0 = 45$ g

(i) Shortening at start

Load (g)	1 9	12 8	23 7	31 9	Mean
Shortening heat (mm)	6 8	8 1	9 2	8 8	8 2

(ii) Release at 1 2 sec

Load (g)	2 7	7 3	12 5	18 3	23 7	31 9	Mean
Shortening heat (mm)	7 1	7 7	8 3	9 0	8 6	9 2	8 15

Exp 34 Temp 0° C Isometric tension $P_0 = 44$ g Release at 1 2 sec Two series, with washing between

Load (g)	3 0	13 9	24 9	Mean
(1) Shortening heat (mm)	6 4	7 2	8 2	7 3
(2) Shortening heat (mm)	7 2	8 0	8 4	7 9

Exp 35 Temp 8 9° C Isometric tension $P_0 = 41$ g Release at 0 4 sec

Load (g)	7 1	18 1	24 6	Mean
Shortening heat (mm)	5 5	6 2	6 1	5 9

Summary

Load (g)	1–10	10–20	20–30	30+
Shortening heat average % of mean	89	102	108	104

The general conclusion is borne out by a considerable number of other incidental results For example (i) the total heat rate has been found to increase linearly with the rate of shortening (varied by altering the load), (ii) the shortening heat, when determined for a larger load but not for a smaller one, has been found to be of the usual order of size as given in Table III (iii) in one experiment at 0° C, for some unknown reason, no allowance was necessary for difference of heat rate, short and long in this experiment the following total deflexions were observed at 2 0 sec , for 5 6 mm shortening, release at the start

Isometric 31 1 mm

Isotonic 2 5 g , 37 9 mm 8 0 g , 39 5 mm , 16 2 g , 38 5 mm , 24 4 g , 38 1 mm

The cumulative effect of this evidence is rather convincing, and as a first approximation at least we may conclude that the heat for shortening is independent of the load, the speed and the work

(iii) *The rate of energy liberation as a function of load* It is now established that, in shortening a distance x, extra heat is liberated equal to ax g cm The work done is Px g cm the total excess energy liberated is $(P+a)\,x$ the rate of excess energy liberation therefore is $(P+a)\,v$, v being dx/dt

In a number of experiments, with a series of different loads, with a already determined (as above) by thermal measurements, and with v read off directly from the mechanical records, the quantity $(P+a)\,v$ was plotted against P with the result shown, for the last seven experiments, in fig 11 A linear relation clearly exists between the energy rate and the load, both at 0° C and at higher temperatures Thus

$$(P+a)\,v = b(P_0-P),$$

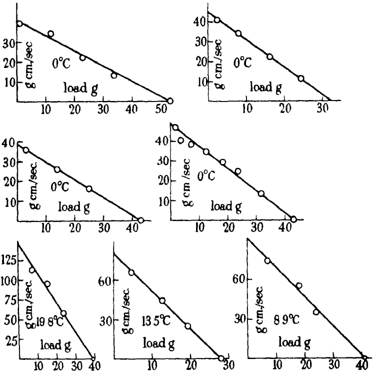

FIG 11 Relation between excess energy liberation per second and load From the last seven experiments made, without selection Horizontally, total load lifted = P Vertically $(P+a)\,v$ where a was calculated from the heat of shortening $(=ax)$ observed in the same experiment, and $v\,(=dx/dt)$ was the speed of shortening observed under load P

where b is a constant and P_0 is the isometric tension The values of b obtained in the present research are given in Table III The temperature coefficient for 10° C is about 2 05

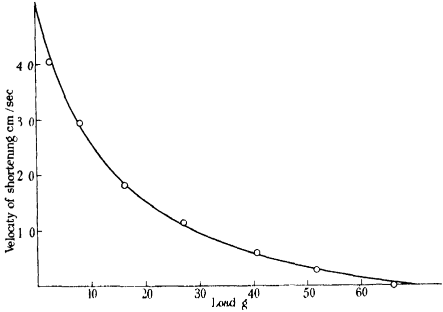

FIG 12 Relation between load (g wt) and speed of shortening (cm /sec) in isotonic contraction Each circle is the mean of two observations in a series and reverse Muscle, 165 mg , 38 mm , in Ringer's solution at 0 C Tetanus 11 4 shocks/sec observations at 4 min intervals 5 6 mm shortening The curve is calculated from the equation $(P+14\ 35)\ (v+1\ 03) = 87\ 6$ Hence $a = 14\ 35$ g wt = 357 g /sq cm , $a/P_0 = 0\ 22$, $b = 1\ 03$ cm /sec = 0 27 length/sec

(iv) *The relation between load and speed of shortening in an isotonic contraction* The linear relation described in the preceding section involves one thermal measurement, that of the shortening heat from which a is derived, and the proof, by thermal measurements, that a is the same for different loads The resulting equation however written $(P+a)\ (v+b) = \text{const}$ can be verified by purely mechanical measurements and the constants a and b can be derived, without thermal measurements at all, by fitting the equation to the observations of P and v It is important that the values of a and b so derived are similar to those directly obtained by the method already described in particular that, when observations by both methods are available, the value of a deduced from the shortening heat is the same as that obtained by fitting the equation to the P-v measurements

An example of the relation between speed and load in an isotonic shortening is shown by the experimental points in fig 12, together with a curve calculated from the equation with the constants given in the legend The fit is good, and the constants are similar to those of Table III The form of the experimental relation is very like that of Fenn and Marsh (1935) the equation is different, but see p 183 below

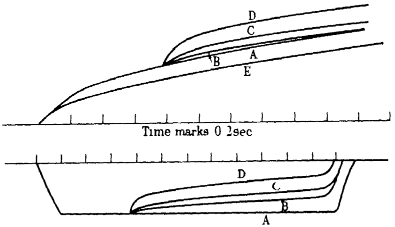

FIG 13 Heat production during lengthening *Below* isotonic contraction 5 75 mm under 2 5 g load then *A* 'isometric short' 40 g tension *B* loaded at about 0 8 sec with 51 7 g *C* with 57 2 g *D* with 62 6 g *Above* corresponding heat deflexions also *E*, 'isometric long' traced from actual records and superimposed Note that in later stages *B* *C* and *D* have a rather less slope than *A* and about the same as *E* Tetanus, 2 sec 0° C muscle 30 mm long 97 mg , time 0 2 sec

A further investigation of the relation between load and speed is being made by Katz and by Baier on the muscles of frogs, tortoises and marine animals, in order to obtain a wider survey of the dynamic constants of muscle

The greatest velocity of shortening viz at zero load from the equation, is bP_0/a With the mean constants of Table III for the frog's sartorius this is 0 331/0 257 = 1 29 × (muscle length)/sec with a temperature coefficient of about 2 05 for 10° C The greatest rate of extra energy liberation is also with zero load its mean value at 0° C is $a_{\max}$ = 399 × 1 29 g cm /c c × sec = 399 × 1 29 × 0 95 g cm /g × sec = 490 g cm /g × sec = 11 5 × 10^{-3} cal /g × sec This is about 4 times as large as the isometric heat rate

(v) *The heat and energy liberation during lengthening* Typical heat and mechanical records, for a muscle lengthening under a load rather greater than the isometric tension, are given in fig 13 The "isometric short" tension was about 41 g , the "isometric long" tension, not recorded, was probably somewhat larger The heat rate, "isometric short" was rather greater than "isometric long", which is a complication in interpreting the results of lengthening

The immediate effect of subjecting a frog's muscle to a load greater than it can bear is to cause it to lengthen, rather rapidly at first, and then, if the load is not too great, more slowly The first more rapid lengthening is "irreversible", in the sense that the energy of the load is largely turned into heat, and a quick upstroke of the heat record occurs If the load is too great (*F*, fig 14 below) the muscle rapidly "gives" the contractile state so to speak collapses, and the heat record (not given in fig 14) shows a large and sudden rise rather like that (fig 8 above) in ordinary relaxation with a heavy load

Gasser and Hill (1924, p 411) found that the quick stretch of a tetanized frog's muscle did not cause the tension to rise suddenly but only slowly after the stretch to the value corresponding to the greater length They suggested that some structure produced or modified by stimulation, "yielded" under a sudden rise of tension With the longitudinal muscle of the body wall of *Holothuria* (Hill 1926*b*) a quick stretch produces the expected rise of tension the converse of the fall of tension on release apparently this muscle does not so readily "give" on stretching Levin and Wyman (1927) found large increases in tension on stretching the muscles of dog fish, *Holothuria* and *Echinus* but only small increases with those of tortoise and crab Some muscles apparently "give" more readily than others

With a smaller load, after the first "irreversible" lengthening the muscle proceeds to lower the load by a process apparently similar to, but the converse of, ordinary isotonic shortening During this phase the energy of the load does not reappear as heat, and the heat record runs approximately parallel to that of an isometric contraction In curves *B* and *C*, fig 13, the muscle, while lengthening slowly, appears to give out heat at a rate definitely less than "isometric short", about the same as "isometric long", slightly less than would correspond to the isometric rate at the intermediate length at which it is at any moment This slight deficit, if genuine, is to be attributed to the negative heat of lengthening, the converse of the positive heat of shortening It occurs so slowly, however, and is so small, that it has proved impossible to measure it, or even to be quite sure of its existence The process cannot be made to occur more quickly (at any rate in frog's muscle) without increasing the load, and that causes "irreversible" lengthening which disguises the whole effect

There is no doubt, however, of the main conclusion, viz that the rate of energy liberation by the muscle is considerably diminished during lengthening under a load In fig 14 the heat record (*C*) of the muscle lowering a load appears, as in fig 13, to rise slightly less rapidly than the isometric heat records, either long (*A*) or short (*B*), due to the negative heat of lengthening From the observed heat has been subtracted the work done by the load on the muscle The difference is shown by D The immediate drop is due to the fact that part of the energy of the load is instantly used to stretch the elastic component of the muscle After that, D rises considerably more slowly than A or B throughout its course But D represents the total energy given out by the muscle, which—as is seen—is much less in lowering a load

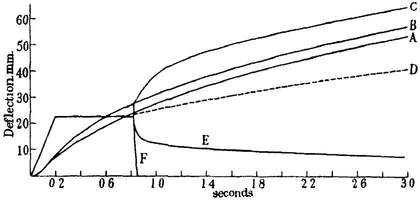

FIG 14 Heat production during lengthening A heat record of isometric contraction (' isometric long) B heat record of shortening 5 6 mm under 2 2 g , followed by "isometric short" B is above A because of the shortening heat C, heat record of contraction similar to B, but loaded at 0 82 sec with 58 6 g E, mechanical record of shortening under 2 2 g , followed by lengthening under 58 6 g Note the rapid fall at first, accompanied by a rapid heat deflexion (C), and the slow lengthening later, accompanied by a heat rate slightly less than in A and B D, net energy liberated during lengthening i e (heat) – (work) note that this rises much more slowly than B and C F, sudden "give" of muscle under 68 2 g , accompanied by a very large and sudden heat deflexion (not shown) Each record is the mean of a pair, in series and reverse Tetanus, 3 sec , 0° C, muscle 29 mm long, 89 mg

This experiment has been made many times, and the result is always the same unless the load is so great that immediate "irreversible" relaxation occurs The linear relation, therefore, shown in fig 11 above between energy rate and load, can be continued to the right, i e to loads greater than the isometric tension, and to negative rates of extra energy liberation No attempt has been made to verify the relation quantitatively in this region, but this might possibly be done It should not be too difficult to get indirect evidence by continuing the experimental relation between load and speed (fig 12 above) to loads greater than isometric, to see whether the equation $(P+a)(v+b) = \text{const}$ applies also to the extension

PART III THE VISCO-ELASTIC PROPERTIES OF ACTIVE MUSCLE

The idea of muscle "viscosity" was proposed, in its modern form, by Hill (1922) to account for the decrease of force with increase of speed in the shortening of human arm muscles This relation between force and speed of shortening does not depend upon any intervention of the nervous system, it is an intrinsic property of muscle itself, as Gasser and Hill (1924) found with isolated muscles and many have found since Whether it is due to "viscosity" or not, this decrease of force with increase of speed is one of the major factors in the dynamics of muscle

In a general way many of the facts had long been known Fick, for example, was aware that a tetanized muscle did less work if it shortened more quickly against a smaller mass, and had supposed (1882, p 67) that a greater part of the energy of its elastic forces was then used in overcoming internal resistances and degraded into heat These internal resistances, he wrote (p 130), were naturally greater the more rapid the movement Blix (1893), to account for after-extension, likened the resting muscle to a spring moving in a viscous fluid There had been, nevertheless, no very precise formulation of the problem, and little attention had been paid to it Since 1922, however, much work has been done, and the term "visco-elasticity of muscle", although implying a theory which is certainly imperfect, has served at least to provoke many new experiments and to focus attention on a very real and important problem

It may be useful, since a rather drastic change is now being proposed in our view of the 'visco elasticity' of muscle, to refer here to papers published in the last 16 years on the subject

A *On human muscle* Hill (1922, 1926*a* 1934*c*) Hansen and Lindhard (1923), Lupton (1922, 1923) Furusawa, Hill and Parkinson (1927) Best and Partridge (1928), Dickinson (1928, 1929) Muller (1930), Fenn (1930*a*, *b*, 1931*a* *b*, 1932), Fenn, Brody and Petrilli (1931), Fenn and Garvey (1934)

B *On isolated unstriated muscle* Hill (1926*a*, *b*) Winton (1930, 1937), Levin and Wyman (1927), Sulzer (1928, 1929), Bozler (1930 1931, 1933 1936), Bayliss, Boyland and Ritchie (1930), van Overbeek (1931) de Nayer and Bouckaert (1932), Postma (1933), Jordan (1933), Bouckaert and Delrue (1934) Shukow (1936)

C *On isolated striated muscle* Gasser and Hill (1924), Hill (1926*a*, 1931*b*), Levin and Wyman (1927), Bouckaert, Capellen and de Blende (1930), Petit (1931), Oinuma and Hayashi (1932), Cattell and Edwards (1932), Stevens and Snodgrass (1933), Stevens and Metcalf (1934), Brown (1934), Fenn and Marsh (1935), Fenn (1936), Hursh (1938)

D *General* Weber (1933, p 139), Jordan (1935)

E *On the effect of work and shortening on the heat production of muscle* Fenn (1923, 1924), Azuma (1924), Hartree (1925, 1928), Hill (1925, 1930), Wyman (1926), Hartree and Hill (1928*a*, *b*), Martin (1928), Cattell (1932), Feng (1932), Fenn and Latchford (1933)

The following facts are clear

(i) active muscle, at least as ordinarily prepared and used, contains an undamped elastic element if, during maintained isometric contraction, the muscle be instantaneously released, the tension does not fall to zero unless the amount of release be sufficient to allow the undamped element to reach its natural length,

(ii) active muscle contains an apparently damped element, in series with the undamped elastic one, the former, if allowed to shorten, exerts a force which is smaller the faster the shortening, until at a certain speed it exerts no force at all at more than that speed it cannot shorten, conversely, if made to lengthen, it exerts a force which is greater the faster the lengthening, until at a certain speed it "gives" like a wire stressed beyond the elastic limit,

(iii) resting muscle contains the elastic element (i), but only to a minor degree the apparently damped element (ii),

(iv) the active state is set up much more rapidly than the isometric tension, as is shown by applying a quick stretch at various moments after a stimulus

Various suggestions have been made to account for the facts The first and obvious one that muscle is endowed with viscosity", resisting change of shape, was soon found to require that the viscosity should be very much higher during activity than at rest—at least in striated muscle It was unlikely, therefore, that simple viscosity alone was involved more likely, that a molecular rearrangement had to occur when an active muscle altered its shape Fenn, however, in 1923, had found that when a muscle shortened and did work it gave out more total energy than when it remained isometric he has several times insisted that this—called by others the Fenn effect—is probably the basis of the apparent viscosity In a recent paper (Fenn and Marsh 1935) he showed experimentally that the relation between speed of shortening and load in an isotonic contraction cannot be deduced from the simple hypothesis of a two-component, (undamped elastic)-(damped elastic) system He found, however, satisfactory agreement with an equation which assumed that the true internal muscle force fell off exponentially as the velocity increased Why it should do so he could not explain, but he argued that the rate of development of "shortening energy" from chemical potential energy is of paramount importance in determining how fast a muscle can shorten under a given load Less clearly defined, his view was much the same as the present one

The relation found experimentally by Fenn and Marsh between speed of shortening and force exerted in an isotonic contraction is the same as that shown in fig 12, to which, as we have seen, the equation

$$(P+a)(v+b) = \text{a constant}$$

applies Their only experiment at 0° C of which details are given is in their Table I, p 284, and the equation

$$(P+450)(v+0\ 35) = 870$$

gives a very satisfactory fit * The values $a = 450$ g /sq cm and $b = 0\ 35$ length/sec agree well with those found on English frogs here Fenn and Marsh give mean values of the constants of their equation, for frog's sartorii at 0° C and at 23° C Calculating a mean curve from these constants, it is possible to fit the equation $(P+a)(v+b) = \text{const}$ to the curve, at 0° C with considerable accuracy, at 23° C with sufficient Numbers recalculated from the present equation and plotted in Fenn's way lie on good straight lines The equations are not the same, but the experiments on shortening alone cannot decide between them The values of the constants a and b which give the best fit are

$$0°\text{ C} \quad a = 640 \text{ g /sq cm}, \ b = 0\ 59 \text{ length/sec}$$
$$a/\text{mean } P_0 = 0\ 38$$
$$23°\text{ C} \quad a = 740 \text{ g /sq cm}, \ b = 1\ 82 \text{ length/sec}$$
$$a/\text{mean } P_0 = 0\ 26$$

Without the original data this roundabout calculation from the means, via Fenn's equation, cannot be very precise, but the result is satisfactory in three ways

(i) in giving values of a and b of the same order of size as have been found in London on different frogs,

(ii) in showing little effect of temperature on a/P_0, and

(iii) in showing a large increase of b with rise of temperature

It is clear, therefore, that the results of Fenn and Marsh are entirely consistent with those recorded above, and that the simpler equation $(P+a)(v+b) = \text{const}$ may be regarded as correctly describing the shortening of muscle under constant load The great virtue of constant load is simply that it eliminates one variable, it avoids change of length

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* Professor Fenn, in a private communication, states that all the velocities and shortenings with his frog lever are given twice as great as they ought to be

of the undamped elastic component If load be not constant, then the elastic element will vary in length as the load varies to it we can then apply the ordinary rules for elastic bodies To the contractile component (better so-called than "damped elastic") the characteristic equation still applies, P and v no longer being constants in any contraction, but functions of the time related to one another by the characteristic equation The apparent "damping" of the contractile component lies in those factors which determine its characteristic equation, viz , as Fenn has maintained, in the manner in which the energy supply is regulated

The experiments on heat and energy in lengthening (figs 13 and 14 above) make it impossible any longer to regard "viscosity" as the primary cause of the effects observed in active muscle During lengthening, at not too high a speed, the rate of heat production is certainly not greater than in an isometric contraction and is probably slightly less If "viscosity" were the chief reason for the slow lengthening, it would undoubtedly be greater The rate of energy liberation by a muscle is considerably diminished during lengthening, considerably increased during shortening these facts find no explanation in the "viscosity" hypothesis, but themselves provide an explanation of the other facts on which the "viscosity" hypothesis was based As Fenn has pointed out, simple viscosity should provide a linear relation between speed and load it has long been recognized that the actual relation is not linear It was always possible, however, in a complex histological structure like muscle, and with a "viscosity" which might really depend on the reorganization of the protein molecules in new crystalline patterns as the muscle altered its shape (Hill 1931*b*, p 120), that a less simple relation between load and speed might be found The "viscosity" hypothesis, therefore, must be dismissed, not for these reasons but because it gives no explanation of the large alterations of heat and energy liberation during shortening and lengthening, and because, from those alterations themselves can be deduced, as a necessary consequence, the facts which the "viscosity" hypothesis was intended to explain The muscle may still have visco elastic properties, but they are less important in its behaviour than the other properties of shortening heat and energy regulation which we have discussed

The general conclusion, therefore, is plain The active muscle is still a two-component system, consisting of an undamped purely elastic element, in series with a contractile element governed by the characteristic equation $(P+a)(v+b) = \text{const}$ There may be, indeed there must be, visco-elastic and probably purely viscous elements as well Convincing evidence for these, in the resting—at any rate in the dead—muscle, is found in the irreversible thermo-elastic effects (Hill and Hartree 1920*b*, Feng 1932, Meyer and Picken 1937) and perhaps in the phenomena of prolonged after-extension (cf Winton 1930) Their contribution, however, is small in the active striated muscle, compared with that of the two main components in the unstriated muscle genuine viscous and visco-elastic factors may conceivably be more important The active striated muscle, however, is chiefly a contractile element governed by the characteristic equation, in series with a purely elastic element

It is not necessary here to attempt to show how all the so-called visco-elastic properties of striated muscle can be derived from the conception of a two component system many of them obviously can others, particularly in the case of twitches or short tetani, require special assumptions as to the manner and speed of disappearance of the active state of the contractile component when a stimulus ends The maintained contraction is simpler Three examples of that are of special interest

(*a*) the form of the isometric contraction, the rate of development of which can be deduced quantitatively from known values of a and b and of c, the amount of stretch of the elastic component,

(*b*) the form of the force-distance curves (Levin-Wyman), during shortening at constant speed,

(*c*) the greatest rate of doing work

(*a*) *The isometric contraction* Assume that the contractile component, of initial length l_c, stretches the elastic component, of initial length l_e, the subsequent lengths at time t being y_c and y_e respectively Assume further

(i) that the active state of the contractile component, defined by the equation $(P+a)(v+b) = \text{const}$, is set up instantaneously on stimulation, and maintained by a tetanus Gasser and Hill (1924) showed that it is certainly set up very rapidly,

(ii) that the elastic component is perfectly elastic, so that the force P is proportional to the stretch $(y_e - l_e)$, i e $P = \alpha(y_e - l_e)$, where α is constant, and

(iii) that the amount of shortening of the contractile component is small enough to allow P_0, the full tension at any of the lengths involved, to remain sensibly constant

In an isometric contraction the rate of shortening of the contractile part is equal to the rate of lengthening of the elastic part, so that

$$v = -dy_c/dt = dy_e/dt = (dP/dt)/\alpha$$

Substituting for v in the characteristic equation and multiplying by α, we obtain

$$(P+a)(dP/dt+\alpha b) = \text{const} = (P_0+a)\,\alpha b \qquad (1)$$

Consider the equation

$$\log_e\left(\frac{1}{1-P/P_0}\right)-\frac{P/P_0}{1+a/P_0}=\frac{t\alpha b/P_0}{1+a/P_0}$$

This is a solution of equation (1), as is seen by differentiating, and it satisfies the initial and final conditions that $P=0$ at $t=0$ and $P=P_0$ at $t=\infty$. Hence it represents the form of the isometric contraction, i.e. P as a function of t. The right-hand side can be simplified, since $P_0/\alpha=$ the amount of stretch of the elastic component under the full isometric tension $=c$. Replacing natural by common logarithms the equation then becomes

$$\log_{10}\left(\frac{1}{1-P/P_0}\right)-\frac{0.4343P/P_0}{1+a/P_0}=\frac{0.4343tb/c}{1+a/P_0} \tag{2}$$

Now we have seen that a/P_0 is approximately constant from one muscle to another and from one temperature to another, so that for frog's skeletal muscle $(1+a/P_0)$ is usually about 1.25. At 0° C the mean value of b is about 0.35 (length)/sec. and c depends on the amount of tendon, on the force developed and on the elasticity of the lever used to record the response: according to Gasser and Hill c is about 0.1 to 0.15 × (length). We have found it usually rather less: for the sake of calculation let us take $c=0.1\times$ (length). Then for the frog's sartorius at 0° C the isometric tension should develop according to the equation

$$\log_{10}\left(\frac{1}{1-P/P_0}\right)-0.3474P/P_0=1.216t$$

For 16° C, assuming a/P_0 to be the same, b to be increased three times and c to become 0.12 (the force exerted being rather greater), the left-hand side of this equation remains unaltered, the right-hand side becomes $3.04t$. In fig. 15 two curves are drawn, for 0° and 16° C, from these equations. They are exactly similar to isometric contractions experimentally observed.

Direct evidence for the agreement of calculated and observed isometric contractions will be given in a later paper by Katz.

Gasser and Hill (1924) and Hill (1926*b*), from their experiments on quick releases during maintained contraction, concluded that the "process determining the speed of redevelopment of tension after release is identical with that determining the speed of initial development of tension". According to the present theory this is necessarily the case, for in both, i.e. in initial development and in redevelopment of tension, the contractile component has to shorten against the increasing force of the elastic component. Apart from the fact that P_0 might be rather less in redevelopment at a shorter length—which would alter c—the equations would be identical

A simple test of the theory was made by taking advantage of the fact that, in equation (2), c can be increased arbitrarily at will by placing an elastic connexion between the muscle and the lever If c is increased, t will be increased in the same proportion and the contraction, remaining exactly similar, will be slowed This was easily verified Further, by increasing c by a known amount and finding the ratio of the times for a given development of tension, the absolute value of c can be determined without a quick release These experiments will be described in more detail elsewhere by Katz

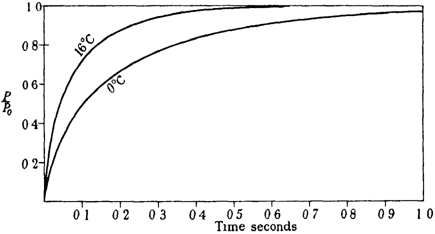

FIG 15 Isometric contractions of frog's sartorius at 0° and 16° C, calculated from equation (2) with the constants given in the text

The shortening of the contractile component and the lengthening of the elastic component in an isometric contraction should have two thermal consequences which are readily verified (cf p 167 above)

(i) during relaxation the stretched elastic component should shorten against the falling tension of the contractile component, and its potential energy should be transformed into heat,

(ii) during the initial development of tension the contractile component should liberate extra heat for shortening, which would exaggerate the early rate of heat production

As regards (i), assuming perfect elasticity, the potential energy is $P_0c/2$, which if $c = 0\ 1 \times$ length becomes $0\ 05P_0 \times$ length In a typical muscle of 100 mg, 3 cm long, developing a force of 60 g, this is 9 g cm, or $1\ 9 \times 10^{-3}$ cal /g, a fairly considerable amount The "hump" on the heat record which regularly occurs in relaxation, at the time when the tension is falling, is just of this order of quantities The whole of the relaxation heat does not occur in this hump, but a large part of it does According to Hartree and Hill (1928*b*, p 12) the relaxation heat after a tetanus is about 0 $12P_0 \times$ length, and about one-half of this must have been derived from the potential energy of the stretched elastic component The remainder is presumably an accompaniment of the disappearance of activity in the contractile component

As regards (ii), if the contractile element shortens by an amount 0 $1 \times$ (muscle length), it should give out shortening heat 0 $1a \times$ (length) In the above example, assuming $a/P_0 = 0\ 25$, $a = 15$ g , so the shortening heat comes to 4 5 g cm or 0 95×10^{-3} cal /g This is large enough to be an important factor in causing the early high rate in the analysis of the heat production of an isometric contraction

The matter can be put more generally Let the contractile portion of the muscle shorten by nl, stretching the elastic portion where n is a fraction and l is the total length of the muscle The total extra energy liberated will be nla as heat for shortening, plus $nlP_0/2$ as work total $nla + nlP_0/2 = nla(1 + P_0/2a)$ If $a/P_0 = 0\ 25$, this becomes $3nla$ Taking a as 400 g /sq cm (mean value for frog's muscle at 0° C), this is $1200nl$ g cm /sq cm $= 1200n$ g cm /c c $= 27n \times 10^{-3}$ cal /g If $n = 0\ 1$, this is 2 7×10^{-3} cal /g of which $\frac{1}{3}$ is shortening heat appearing early in contraction, $\frac{2}{3}$ is work done in stretching the elastic component, which appears as heat in relaxation

The ratio, in an isometric contraction of "shortening heat" to "relaxation hump" is 1 $P_0/2a$, 1 2 on the average, and does not depend on the actual amount of elastic shortening

(*b*) *The force-distance relation at constant speed* Consider a muscle tetanized isometrically till its full tension is developed, and then released at constant velocity with the Levin-Wyman apparatus With the same symbols as for (*a*) above and u = velocity of shortening, we have

$$u = -dy_c/dt - dy_e/dt = \text{constant},$$

$$v = -dy_c/dt,$$

$$P = \alpha(y_e - l_e),$$

$$-dy_c/dt = u + dy_e/dt = u + (dP/dt)/\alpha$$

Substituting in the characteristic equation and multiplying by α,

$$(P + a)\,(dP/dt + \alpha u + \alpha b) = \text{const} = (P_u + a)\,\alpha(u + b), \tag{3}$$

where P_u is the steady force corresponding to velocity u (It is assumed that the amount of shortening is not so great that P_0 sensibly changes)

This is the same as equation (1) above with $(u+b)$ instead of b and P_u instead of P_0. The boundary conditions are now, (i) at $t=0$, $P=P_0$, and (ii) at $t=\infty$, $P=P_u$. Note that P_u can be calculated from the characteristic equation, $(P_u+a)(u+b)=(P_0+a)b$.

Consider the equation

$$(P_0-P)+(P_u+a)\log_e\left(\frac{P_0-P_u}{P-P_u}\right)=P_0(b+u)t/c, \tag{4}$$

where c, as before, is the amount of stretch of the elastic component under the full isometric tension P_0. This is a solution of equation (3), as is seen by differentiating and it satisfies the initial condition that $P=P_0$ at $t=0$, and the final condition that $P=P_u$ at $t=\infty$. Hence it represents the relation between P and t during shortening of the whole muscle at constant velocity u.

Consider the case of a frog's sartorius at 0° C, for which $P_0=100$ g., $a=25$ g., $b=1$ cm./sec. and $c=0.25$ cm. and let us calculate a family of "indicator" curves, similar to those of Levin and Wyman (1927), for a set of velocities $u=0.25$, 0.5, 1, 2, 3 and 5 cm./sec. The result is shown in fig. 16. It is obviously similar to such sets of curves as were given in figs. 2, 6 and 10 of Levin and Wyman's paper. In actual practice the amount of shortening necessary before a steady force is reached is so large that P_0 is less than at the start. The curve for $u=0$ in fig. 16 is horizontal: in practice it will slope downwards somewhat from the left. This slight complication, however, does not disguise the main effect, viz. that the general form of the indicator curves, for a set of constant velocities, can be deduced from the theory of the two-component system, with values of a, b and c found by independent methods.

Details of experiments made with the Levin-Wyman apparatus to test the theory will be published elsewhere by Shapiro.

(*c*) *The greatest rate of doing work.* The rate of doing work is Pv, which from the characteristic equation becomes $bP[(P_0+a)/(P+a)-1]$. This can be shown to be a maximum when $P/a=\sqrt{(1+P_0/a)}-1$. The usual value of a/P_0 in the frog's sartorius is about 0.25: inserting this, $P/a=\sqrt{5}-1=1.24$, or since $P_0/a=4$, $P/P_0=0.31$: thus the greatest rate of doing work should occur with a load equal to about 0.3 of the full isometric tension. For different values of a/P_0 in the neighbourhood of the mean, the value of P/P_0 giving the greatest work rate varies only slightly.

a/P_0	0.15	0.20	0.25	0·30	0.35
Optimum P/P_0	0.27	0.29	0.31	0.32	0.34

Thus in any practical case the greatest rate of doing work will occur with a load about $\frac{3}{10}$ of the maximum force This may have practical applications

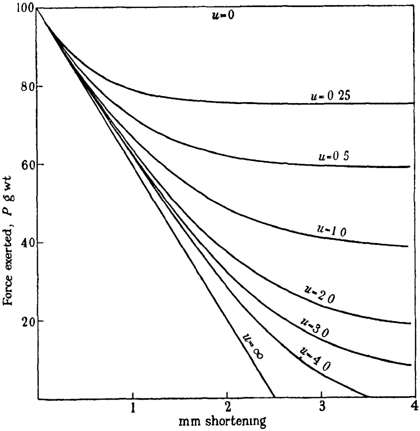

Fig 16 Force distance curves, for constant speeds of shortening during a fully developed tetanus, as made by the Levin Wyman apparatus, calculated for 0° C from equation (4), with the constants given in the text Speeds given in cm /sec on the curves

The active state of muscle It has generally been assumed that the simplest "unit" of muscle activity is the twitch, and that all maintained activity can be regarded as due to the fusion or summation, partial or complete, of twitches This view has something to commend it from the point of view of excitation, but from that of energy exchange it is simpler to think of muscle as having two states, that of rest and that of maximal maintained activity During rest the source of energy is cut off completely from the contractile mechanism During a tetanus of sufficient frequency the barrier is removed and the two are steadily connected energy is set free in maintaining the active state, and if shortening occurs extra energy is liberated for shortening and as mechanical work When the stimulus ends the barrier begins to fall, there is a rapid decrease in the rate of heat production, and in a short time the contractile mechanism is again isolated from the energy source

After a single shock the conditions are more complex and the analysis is more difficult The barrier is raised, the active state rapidly sets in, the contractile part of the muscle begins to shorten and stretch the elastic part, an external force is gradually developed, and shortening may occur according to the mechanical conditions The barrier, however, begins to fall again, the energy supply is gradually cut off and the active state disappears The energy relations, heat and work, of a twitch are complicated by the movements of the barrier, i e by a process which has more to do with excitation—or the immediate result of excitation—than with the properties of the active state

For the present purpose, therefore, we have considered a muscle as having two states, (A) that of rest with the low heat production of rest, (B) that of activity with (i) the high heat production of activity, and (ii) the capacity for shortening and doing work governed by the characteristic equation and its corresponding energy relations We have not been concerned with the process of transformation from one state to the other, but have assumed that the active state is set up very rapidly after the first shock—for this there is strong experimental evidence—and that it is steadily maintained by a tetanus of sufficiently high, but not actually very high, frequency

Gasser and Hill (1924) examined the onset and disappearance of the "fundamental mechanical response" in a twitch, by applying a quick stretch at various moments before and during contraction They found that its intensity reaches its maximum very early after the shock, and passes off continuously thereafter The energy liberated in a twitch, as well as in a tetanus, is affected by mechanical conditions existing during contraction (Hill 1930), and a few preliminary experiments with the present equipment have shown that in a twitch in which shortening is allowed the heat is given out earlier than in an isometric one

It is clear that the energy of a twitch is not set free in a single "all-or-none" outburst, but continues to appear at a decreasing rate as the "barrier" is lowered, and is affected by shortening and work as is the energy in a maintained contraction The twitch, however, is much more complex than the maintained contraction and needs to be specially investigated Further exploration of its energy relations may throw light on the manner in which the "energy store" is linked to the "contractile mechanism" during activity

SUMMARY

Part I

1 A coupled galvanometer system is described which is sensitive enough for muscle heat measurements with a deflexion time of 30–50 msec Its sensitivity and period can be regulated by an adjustable feed back Used with a "protected" thermopile 45 μ thick, it is rapid enough to allow the initial heat to be read from the records in absolute units and (for most purposes) without analysis

2 The great advantage, in speed and accuracy, of using a very thin muscle, and the anomalies caused by non-uniform contraction and relaxation, are discussed

3 The possibility is considered of using a resistance thermometer instead of a thermopile Very serious difficulties are foreseen

Part II

4 When a muscle shortens, in a tetanically maintained contraction, it liberates extra energy in two forms, (i) as "shortening heat", in amount proportional to the shortening, and (ii) as external mechanical work The shortening heat is independent of the load, and therefore of the work done and the speed of shortening

5 The rate of energy liberation at any moment, i e of work + heat, is a linear function of the load, increasing as the load diminishes

6 The "dynamic constants" of muscle, in a maintained contraction, are defined and measured They are

a, the shortening heat per cm of shortening,

P_0, the isometric tension,

b, the increase of energy rate per g wt decrease of load

At any temperature these quantities are very constant a/P_0 is independent of temperature, b has a temperature coefficient of about 2 05 per 10° C

7 A "characteristic equation" is deduced, for the speed of shortening v under a load P, viz $(P+a)(v+b) = \text{const}$ This equation is very accurately obeyed By fitting it to observations of v and P, the same constants a and b are obtained as are given by heat measurements

8 When a contracting muscle is made to lengthen gradually by applying a load rather greater than the isometric tension, there appears to be a negative heat of lengthening and the total energy given out by the muscle is less than in an isometric contraction The energy relations in lengthening are the converse of those in shortening and seem to be governed by the same rules

9 The nature of the shortening heat, and the mechanism by which the energy rate is governed by the load are discussed It is suggested that the chemical processes by which energy is liberated involve certain active points in the molecular machinery, and that when these are occupied in attracting one another and producing a tension they are not available to take part in chemical reaction

10 The work done by a muscle in raising a load, or in overcoming any elastic resistance, is transformed into heat in relaxation

11 There is a sudden drop in the heat rate directly the stimulus ends, and before visible relaxation occurs

12 The effect of muscle length on the isometric heat rate is described

Part III

13 The "visco-elasticity" of contracting muscle is shown to be the property of a two component system, the one component being undamped and elastic, the other being governed by the characteristic equation $(P+a)(v+b) = \text{const}$ and its corresponding energy relations The fact that an active muscle shortens more slowly under a greater force is due, not to "viscosity" but, as Fenn has claimed, to the manner in which the energy liberation is regulated A large force causes a low energy rate, which results in a low speed

14 From this can be deduced (i) the form of the isometric contraction, (ii) the form of the force-distance curves made, at constant velocity, by the Levin-Wyman apparatus, and (iii) that the greatest rate of doing external work should occur with a load equal to about 30 % of the isometric tension

15 The active state of muscle is discussed

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The distribution of carbon dioxide in the hen's egg

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The presence of bicarbonate in egg-white was reported by Scholl (1893) His suggestion that egg-white resembles blood serum is in accordance with the pH values given in Table I Healy and Peter (1925) found a value of pH 8 2 on laying and suggested that fresh white contained both bicarbonate and carbon dioxide

Table I

pH of egg white			
On laying	After storage in air	Method	
8 2	9 7	Colorimetric	Healy and Peter (1925)
8 2	9 8	Colorimetric	Buckner and Martin (1929)
7 83	9 45	Hydrogen electrode	Romanoff and Romanoff (1929, 1933)
7 6	9 7	Hydrogen electrode	Sharp and Powell (1931)
7 97	9 2–9 5	Glass electrode	Bate Smith (1934)
	9 2–9 7	Glass electrode	Moran (1937)
		pH in 100 % CO_2 6 4	Healy and Peter
		6 5	Moran
		10–12 % CO_2 7 6	Sharp (1929)
		3–4 5 % CO_2 7 9	Bate Smith (1935)

Apart from the pH measurements, little is known of the relation between CO_2 and the constituent phases of the egg The pH of the white depends on the partial pressure of CO_2 and ranges roughly from 9 7 in air to 6 5 in 100 % CO_2, the pH of the infertile yolk was found by the authors in Table I to be 6 0–6 3 and changing the CO_2 pressure had little effect on the pH

In the present work the total CO_2 in egg-white was measured by the method of van Slyke and Neill (1924) and the combined CO_2 by the manometric method of Warburg (1925) The combined CO_2 is the aggregate of bicarbonate, carbamino, and unknown forms in which CO_2 is bound to protein It is provisionally assumed that all the combined CO_2 in the white is in the form of bicarbonate, direct evidence for the absence of carbamates would also require (cf Roughton 1935) an accurate knowledge of the pH and the effect of proteins on the activity coefficient of the bicarbonate ion or, alternately, an investigation of the kinetics of the reaction The assumption leads to reasonable values for the protein dissociation and the pH over a wide range of CO_2 pressures and therefore gives indirect evidence that, as in blood serum, the carbamino-CO_2 is relatively small in amount

THE BICARBONATE CONTENT OF EGG-WHITE

Duplicate measurements of the combined CO_2* in weighed amounts of the white in equilibrium with different partial pressures of CO_2 at 25° C agreed to within 0 5 %

Material Outer thin white was obtained from the infertile eggs (1–2 days old) of Light Sussex hens The mean density of the white at 25° C was taken as 1 040

Gas mixtures Cylinders containing specially prepared mixtures of air and CO_2 were used The CO_2 contents were determined by the method of van Slyke and Sendroy (1932), the accuracy was 0 05 vol % The outlet stopper of the manometric vessel (Warburg and Kubowitz 1929) permitted equilibration in the thermostat Partial pressures of CO_2 (p) are given in atmospheres

In the calculation of the results the volume of 1 g mol CO_2 (0 °C, 760 mm Hg) was taken, following van Slyke, as 22 261 (Guye and Pintza 1908) Results are given in either g mol /l of white, $[HCO_3^-]_c$, or g mol / 1000 g solvent, $[HCO_3^-]_m$ The two quantities are related by the formula

$$[HCO_3^-]_c = \frac{[HCO_3^-]_m\, d(w - mn)}{100} = \frac{[HCO_3^-]_m}{1\ 125}, \qquad (1)$$

where d = density of white = 1 040, w = g H_2O per 100 g white = 88, m = g dry protein per 100 g white = 11, n = water of hydration in g H_2O per g dry protein = 0 23 The values of w and m are mean values from the literature (Needham 1931) The hydration is assumed to be the same as for crystalline egg-albumin (Sørensen 1917, Adair and Adair 1936), the assumption agrees with Hill's (1930) result for the amount of bound water in the white (about 3 % of the total water)

---

* Excess acid was added to white in equilibrium with a given CO_2 pressure The formula used in the calculation of the results was not appreciably affected by taking the solubility of CO_2 in the white to be the same as in H_2O, since the volume of white (about 0 2 c c) was small compared with the volume of the vessel (about 16 c c) The results were reproducible, no change in concentration was found after keeping samples of white at 0° C for 1 week

The third column of Table II contains the mean values* of the HCO_3^- concentrations (the meaning of the bracketed values is given below) It will be seen that an increase in CO_2 pressure increases $[HCO_3^-]_c$ and that the effect is most marked at the lower pressures The equilibrium between white and CO_2 therefore resembles that between blood serum and CO_2, where the proteins react to the addition of acid by reversible combination with H^+ When the acid is H_2CO_3, the net reaction may be written

$$Pr^- + H_2CO_3 \rightleftharpoons HPr + HCO_3^-, \tag{2}$$

where Pr^- and HPr represent protein anions and undissociated protein respectively †

The HCO_3^- concentration may be treated either as a function of the CO_2 pressure or, more conveniently, of the pH The pH of the white was calculated from equation (3) and the values are given in the fourth column of Table II

$$pH = pK_1 + \log \frac{[HCO_3^-]_m}{Sp} + \log \gamma_{HCO_3^-} \tag{3}$$

The thermodynamic first dissociation constant, pK_1, was taken as 6 37 at 25° C and S, the Henry's law constant for CO_2 and pure water, as 0 0337 mol /l /atm (Shedlovsky and MacInnes 1935) The usual assumption was made that the proteins had no effect on $\gamma_{HCO_3^-}$, the activity coefficient of the HCO_3^- ion (in the case of blood serum this assumption is justified, cf van Slyke, Hastings, Murray and Sendroy 1925, Hastings, Salvesen, Sendroy and van Slyke 1927) The activity coefficient is given by the Debye-Huckel equation

$$-\log \gamma_{HCO_3^-} = \frac{0\ 504\sqrt{\mu}}{1+3\ 28\times 10^7 a\sqrt{\mu}} \tag{4}$$

Kieland (1937) gives the value of a for the HCO_3 ion as $4\ 3\times 10^{-8}$ The ionic strength μ is not known with certainty and was taken as 0 13, the value for a

---

* Each experiment number refers to a different sample of thin white, with the exception of 3b, where thick white from egg 3a was used, the HCO_3^- concentration was greater in the thin than in the thick white Romanoff and Romanoff (1929) found that on laying the pH of the outer thin white was higher than that of the thick white by 0 05 pH As the difference in HCO_3^- concentration accounts for only 0 02 pH units at a given CO_2 pressure, it is possible that on laying the outer thin white contains more HCO_3^- and less CO_2 than the thick white

† Since the work of Hasselbalch (1917) many papers have been published on this system The predominant reactant is protein, other anions in serum, e g phosphate, are relatively unimportant

NaCl solution of the same freezing-point (-0.45° C) as egg-white.* The value of $\log \gamma_{HCO_3^-}$ was then -0.12.

TABLE II. 25° C

Exp.	p atm.	$[HCO_3^-]_c$ mol./l.	pH	$-\Delta[HCO_3^-]_c$	$-\Delta a \times 10^5$
1	0.961	0.0613	6.58		
		(0.0615)	6.55	0	0
	0.0491	0.0542	7.82	0.0073	6.4
3a	0.969	0.0602	6.57		
		(0.0603)	6.55	0	0
	0.0491	0.0534	7.81	0.0069	6.0
3b	0.969	0.0578	6.55	0	0
	0.0491	0.0506	7.79	0.0072	6.3
20	0.962	0.0597	6.57		
		(0.0598)	6.55	0	0
	0.395	0.0573	6.94	0.0025	2.2
28	0.955	0.0551	6.54		
		(0.0550)	6.55	0	0
	0.392	0.0533	6.91	0.0017	1.5
	0.143	0.0512	7.33	0.0038	3.3
38	0.961	0.0582	6.56		
		(0.0583)	6.55	0	0
	0.395	0.0560	6.93	0.0023	2.0
	0.143	0.0540	7.35	0.0043	3.8
	0.0491	0.0512	7.79	0.0071	6.2
43	0.961	0.0597	6.56		
		(0.0597)	6.55	0	0
	0.395	0.0576	6.93	0.0021	1.8
	0.143	0.0552	7.36	0.0045	3.9
	0.0491	0.0526	7.80	0.0071	6.2

When $[HCO_3^-]_c$ was plotted against pH a series of straight lines was obtained, each sample gave a different line but the slope was the same. It was therefore more convenient to plot the change in $[HCO_3^-]_c$ from a common pH. At $p = 0.955$–0.969 atm. the pH of the different samples varied from 6.54 to 6.58, a small interpolation gave $[HCO_3^-]_c$ at pH 6.55. These values are bracketed in the third column of Table II. The fifth column gives the decrease in $[HCO_3^-]_c$, $-\Delta[HCO_3^-]_c$, with increase in pH.

---

* As μ enters into equation (4) in the form $\sqrt{\mu}$, it was unnecessary to correct the freezing point depression of egg white for the small amounts of non electrolyte present. Glucose is the only non electrolyte found in any significant amount (0.45 g./100 g. white, cf. Sørensen 1934).

from 6 55 The value of Δa in the last column is defined by equation (5)

$$\Delta a = \frac{\Delta[\mathrm{HCO_3^-}]_c}{114\ 4}, \tag{5}$$

where 114 4 is g dry protein per litre of white

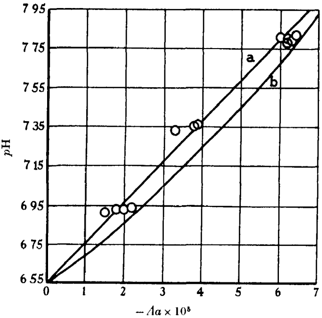

FIG 1 The relation between the pH and the dissociation of (a) egg white proteins, (b) crystalline egg albumin

In fig 1 Δa is plotted against pH all the values fall on the same straight line (within the experimental error) * For comparison is given the smoothed titration curve of crystalline egg albumin over the same pH range determined by Kekwick and Cannan (1936) The quantitative agreement is not stressed since (a) the values of Kekwick and Cannan were determined at low salt concentrations, the only solutes being the protein and the NaOH used for titration, (b) crystalline egg-albumin forms only about 70 % of the total proteins of the white (Sørensen 1934, Taylor, Adair and Adair 1932) Provisionally, however, it may be assumed that the equilibrium between CO_2 and the white is given by equation (2)† over the pH range 6 55–7 82

---

* The experimental error in $\Delta[\mathrm{HCO_3^-}]_c$ or Δa is rather large since it is a relatively small change in a large total concentration

† The concentration of phosphate ions in egg white is negligible (Grossfeld and Walter 1934, Eble and Pfeiffer 1935) No correction was made for the small amount of $CO_3^{=}$ present at $p\mathrm{H} \sim 7\ 8$

The curve for egg-white in fig. 1 is given by equation (6)

$$-\Delta a = 4.75 \times 10^{-5}(p\mathrm{H} - 6.55) \tag{6}$$

The buffer value β per g. protein over the pH range 6.54–7.82 is then

$$\beta = -\frac{da}{dp\mathrm{H}} = 4.75 \times 10^{-5}$$

In Table III is recorded the buffer values of other proteins over a similar pH range. The buffer value of egg white proteins is roughly one half that of serum proteins.

TABLE III

Protein	$\beta \times 10^5$	
Human haemoglobin*	13.7	Adair (1925)
Sheep CO haemoglobin*	11.5	Adair and Adair (1934)
Horse serum	9.6	van Slyke, Hastings,
Human serum	10.4	Hiller and Sendroy (1928)
Crystalline egg albumin	5.2	Kekwick and Cannan (1936)
Egg white	4.8	

* In the region of the iso-electric point.

THE SOLUBILITY OF CARBON DIOXIDE IN EGG WHITE

The dissolved CO_2 in egg-white is given by the difference between total CO_2 (as determined by van Slyke) and combined CO_2. Thin white (about 25 c.c.) was equilibrated at 25° C. with a given pressure of CO_2 using an apparatus previously described (Brooks 1935).

(*a*) samples were withdrawn in a special pipette, 0.5 c.c. introduced into the chamber of the van Slyke apparatus without contact with air and the total CO_2 determined (cf. Peters and van Slyke 1932),

(*b*) the concentration of combined CO_2 was measured at 25° C. by the manometric method of Warburg, using the same gas mixture for equilibration.

In both cases duplicate measurements agreed to within 0.5 %.

The procedure was tested with 0.05 M $NaHCO_3$ at $p = 0.961$ atm. and 25° C. The concentrations of total and combined CO_2 were 0.0822 and 0.0501 mol./l. respectively, the Bunsen solubility coefficient α was therefore 0.744. For pure water at 25° C $\alpha = 0.751$ (Shedlovsky and MacInnes 1935). If the salting-out coefficient K for $NaHCO_3$ is the same as for NaCl, the corrected value[†] of α at $\mu = 0.05$ is 0.744.

[†] From the equation $\log S_0/S = K\mu$ (Randall and Failey 1927). S_0 is the solubility in pure water. S the solubility at ionic strength μ. The value of K for NaCl was obtained from the data of van Slyke, Sendroy, Hastings and Neill (1928) at 38° C. The value of K does not vary with temperature between 25° and 38° C (cf. Walker, Bray and Johnston 1927).

The results in Table IV are the mean of duplicate analyses The values were reproducible, the same sample was analysed over the cycle $p = 0\ 96$–0 40–0 96–0 40 atm and concordant values obtained (Exp 20) The last column gives the Bunsen solubility coefficient

TABLE IV 25° C

Exp	p atm	Mol /l Total CO_2	 Combined CO_2	α
20	0 961	0 0904	0 0597	0 711
	0 963	0 0900	0 0596	0 703
	0 395	0 0702	0 0575	0 716
	0 395	0 0698	0 0571	0 716
28	0 955	0 0859	0 0551	0 718
	0 392	0 0662	0 0533	0 733
	0 143	0 0557	0 0512	0 700

Henry's law is obeyed over the range $p = 0\ 96$–0 14 atm , the best value of α at 25° C is 0 71 The solubility coefficient per g H_2O in the white* is 0 80, the corresponding value for pure water is 0 753—a difference of 6 % Since the salts in the white lower the solubility† by about 2 %, some factor is responsible for a solubility roughly 8 % greater than expected from the salt and water content A similar excess solubility of N_2 in blood serum was found by Stoddard (1926–7) and of CO_2 in blood serum by van Slyke, Sendroy, Hastings and Neill (1928) and attributed to the presence of small amounts of lipoids Egg white apparently also contains some lipoid (Needham 1931, pp 265, 301) but the amount is uncertain

THE pH OF EGG-WHITE

At pH 6 55 the mean value of $[\mathrm{HCO_3^-}]_c$ was 0 058, equations (1) and (3) then give the following mean values $[\mathrm{HCO_3^-}]_m = 0\ 0652$ and $p = 0\ 97$ atm , this may be taken as the standard state‡ of egg-white Considering the volume of white (1125 c c) which contains 1000 g free H_2O (and 128 6 g protein), from (6),

$$\begin{aligned} -\Delta[\mathrm{HCO_3^-}]_m &= 4\ 75 \times 10^{-5}(p\mathrm{H} - 6\ 55) \times 128\ 6 \\ &= 6\ 1 \times 10^{-3}(p\mathrm{H} - 6\ 55) \qquad (7) \end{aligned}$$

---

* c c CO_2 (0° C, 760 mm Hg) dissolved at 25° C and $p = 1$ atm in the volume of white which contains 1 g free water (1 125 c c)

† The salting out coefficient K was taken as 0 077—a value between the coefficients for KCl (0 062) and NaCl (0 086) obtained from the data of van Slyke, Sendroy, Hastings and Neill (1928) at 38° C

‡ The pH of the different whites at a given value of p varied by only 0 04 pH units It is possible that eggs from different sources might show larger variations

The general equation at 25° C for the pH of white between $p = 0.05$–1.0 atm. can then be written

$$pH = 6.25 + \log \frac{0.0652 - 6.1 \times 10^{-3}(pH - 6.55)}{0.0337\,p} \tag{8}$$

When p is less than roughly 0.05 atm. equation (8) no longer holds since the HCO_3^- concentration is lowered by reaction (2) and by the dissociation of HCO_3^- to $CO_3^=$. The governing equation is then

$$-d[HCO_3^-]_m = -da' + 2d[CO_3^=]_m, \tag{9}$$

where da' is the change in g.-equivalents H^+ combined with 128.6 g. protein. If the buffer value of the proteins is assumed to be unchanged above pH 7.8, the conditions which obey (9) and (10) can be calculated, (10) gives the relation between K_1 and K_2, the first and second thermodynamic dissociation constants of H_2CO_3.

$$\frac{[HCO_3^-]_m^2}{[CO_3^=]_m} = \frac{K_1 \gamma_{CO_3^=} S p}{K_2 \gamma_{HCO_3^-}^2} \tag{10}$$

K_2 was taken as 5.61×10^{-11} at 25° C (MacInnes and Belcher 1933) and $\gamma_{CO_3^=}$, the activity coefficient of the carbonate ion, was calculated from the Debye-Huckel equation using Kieland's (1937) value for a (4.5×10^{-8}).

The calculated values for the "mean white" are given in Table V and fig. 2, the partial pressure of CO_2 in air is 0.0003 atm.

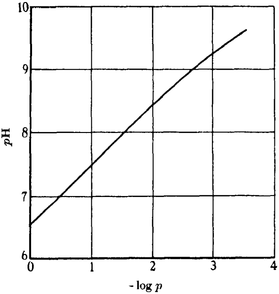

FIG. 2. The relation between the pH of egg white and the partial pressure (in atmospheres) of carbon dioxide.

TABLE V 25° C

p atm	$[HCO_3^-]_m$	$[CO_3^=]_m$	pH
0 97	0 0652	—	6 55
0 10	0 0594	0 0002	7 50
0 05	0 0568	0 0004	7 78
0 03	0 0551	0 0007	7 99
0 01	0 0504	0 0017	8 43
0 0003	0 0231	0 0117	9 61

The pH values in Tables I and V are in rough agreement

THE BUFFER VALUE OF EGG WHITE

The buffer value is defined by van Slyke (1922) as $dB/dp\mathrm{H}$ expressing the relation between the increment (in g -equivalents per litre) of strong base B added to a buffer solution and the resulting increment in pH For egg-white there are two limiting cases

(*a*) no gas phase,

(*b*) a gas phase at a constant pressure of CO_2

Case (*b*) does not strictly correspond with the concept of buffer value, the greater ability of this system to buffer against the addition of non-volatile acids or bases as compared with case (*a*) (cf Table VI) is due mainly to the escape or absorption of CO_2 to or from a gas phase of a constant CO_2 pressure The value of $dB/dp\mathrm{H}$ for this two-phase system may be defined as the buffer capacity βp

It can be shown that for the volume of white which contains 1000 g free water

$$\text{Case }(a)\ \beta = \frac{dB}{dp\mathrm{H}} = -\frac{da'}{dp\mathrm{H}} + \frac{2\ 303[CO_2][HCO_3^-]}{c} + \frac{2\ 303[CO_3^=]}{c}([HCO_3^-]+4[CO_2]), \qquad (11)$$

$$\text{Case }(b)\ \beta p = \frac{dB}{dp\mathrm{H}} = -\frac{da'}{dp\mathrm{H}} + 2\ 303[HCO_3^-] + 4\times 2\ 303[CO_3^=], \qquad (12)$$

where $$c = [CO_2]+[HCO_3^-]+[CO_3^=]$$

da' is the change in g -equivalents H^+ combined with 128 6 g protein, from (6) or (7) $da'/dp\mathrm{H} = 6\ 1\times 10^{-3}$ Other concentration terms are expressed in g mol /1000 g free water Calculated values are given in Table VI, the concentration of dissolved CO_2 corresponds to the CO_2 pressures in the first column

TABLE VI 25° C

	Case (*a*)		Case (*b*)	
p atm	β	% buffering due to proteins	βp	% buffering due to proteins
0 0003	0 024	25	0 167	3 7
0 03	0 010	61	0 139	4 4
0 10	0 014	43	0 145	4 1
0 20	0 021	30	0 149	4 0

Intermediate cases lie between these two extremes, depending on the relative volumes of liquid and gas

Romanoff and Romanoff (1929, 1933) found that the *p*H of the white of the fertile egg increased to 9 5 in 2 days (loss of dissolved CO_2) and then decreased slowly to about 7 2 after 15 days incubation, the cause of this decrease is uncertain (cf Needham 1931 p 1202) Although equations (11) and (12) apply only to equilibrium systems, they indicate that the value *p*H 7 2 may not be representative of the conditions within the intact egg The removal of CO_2 *in vivo* from the blood by the respiratory apparatus (cf van Slyke 1922) is replaced during incubation by factors such as rates of diffusion and ventilation If non volatile acids are produced by the embryo, the extent of the *p*H change in the white (and in the embryonic tissues) will depend on the rate of removal of CO_2

THE UPTAKE OF CARBON DIOXIDE BY SHELL AND YOLK

Shell In spite of the porous structure of the shell* no significant adsorption at 25° C was detected at two pressures of CO_2

(*a*) $p = 0\ 014$ atm CO_2 Coarsely powdered shell (0 25 g) was placed in the central tower of a Warburg manometric vessel and a known amount of CO_2 formed by tipping excess acid from the side bulb into Na_2CO_3 solution in the main vessel The adsorption was calculated from the final pressure increase

(*b*) $p = 0\ 99$ atm CO_2 The usual type of adsorption apparatus was used (containing 6 5 g shell)

The results are given below The CO_2 exchanges of the shell reported in the literature cannot be explained by adsorption

p atm	c c CO_2 (0° C, 760 mm) adsorbed per g shell
0 014	$<0\ 01$
0 99	$<0\ 01$

---

* The structure of the shell has been reviewed by Needham (1931) and Stewart (1935)

Yolk The total uptake of CO_2 at 25° C by the yolk of infertile eggs was measured approximately

(*a*) Yolk was placed in the main compartment of a Warburg manometric vessel * The central tower contained acid and the side bulb Na_2CO_3 solution Tower and side bulb were connected by a bent glass capillary which allowed excess acid to be siphoned into the side bulb when vessel and manometer were tilted The Bunsen solubility coefficient α was calculated from the equilibrium CO_2 pressure (mean $p = 0\ 0136$ atm)

(*b*) A whole yolk was stored *in vacuo* over 0 2 N KOH for 24 hr and placed in the adsorption apparatus The volume of CO_2 required to fill the apparatus to $p = 0\ 96$ atm CO_2 was measured after evacuation Equilibrium was reached in about 60 hr The values obtained are given below

p atm	α
0 0136	1 2
0 96	1 3

With white a value of α calculated from the total CO_2 uptake (dissolved plus "retained" CO_2) would be very different at the two pressures, with yolk the values were roughly the same †

The fats and lipoids in the yolk are probably responsible for the greater solubility of CO_2 in yolk ($\alpha \sim 1\ 2$–$1\ 3$) than in white ($\alpha = 0\ 71$) If allowance is made for the CO_2 dissolved in the aqueous phase of the yolk, the value of α per g of total fatty material is very roughly 3

THE SALTS OF WHITE AND SHELL

The relationship between the salts of the white and the shell is obscure Typical analyses for the infertile egg are given‡ in Table VII, the $CaCO_3$ of the shell is in the form of calcite The system is only defined when in equilibrium with a known pressure of CO_2, it is probable that this condition is never fulfilled Ionic concentrations at equilibrium, corresponding to the known HCO_3^- concentrations, have been calculated approximately† for 25° C and are given in Table VII.

---

* The yolk used did not evolve CO_2 during the experiment

† The CO_2—HCO_3^-—$CO_3^=$ system appears to play little or no part in the buffering of the yolk, the same conclusion may be reached from the pH of the yolk (about 6 2 in air) or the analyses of Bialascewicz (quoted by Needham 1931 Table 47) of the ultra filtrate of yolk

‡ The values for the white have been recalculated on the basis that 100 g white contain 85 5 g free H_2O Langworthy's and Iljin s results are quoted by Needham (1931, Tables 9 and 27) Straub and Donck (1934) give the HCO_3^- concentration of white as 0 0343 mol l of white at apparently pH 8, this value is very different from those obtained in the present work

TABLE VII

Shell

% $CaCO_3$	$MgCO_3$	$Ca_3(PO_4)_2$	Organic material	
93.7	1.3	0.8	4.2	Langworthy (1901)

White

Millimols per 1000 g. H_2O

Ca	Mg	Cl	
2.8	4.1	51.5	Iljin (1917)
4.0	7.7	51.5	Straub and Donck (1934)

Calculated equilibrium concentrations at 25° C, millimols per 1000 g. H_2O

p atm	HCO_3^-	Ca^{++}	Mg^{++}	Total phosphate ions
0.97	65.2	1.2	1900	0.15
0.10	59.4	0.15	230	0.2
0.05	56.8	0.1	130	0.2
0.0003	23.1	0.001	5*	3.0

* Solid phase $Mg(OH)_2$

The CO_2 "pressure" in the oviduct of the hen is unknown, but the pH of the white before laying (found by Romanoff and Romanoff (1929) to be 7.6) corresponds roughly to 0.10 atm. CO_2 at 42° C (the body temperature of the hen). The Ca content of the white,‡ however, is even greater than the calculated Ca^{++} concentration at $p = 0.97$ atm.: this may be partly due to the presence of undissociated Ca compounds. The concentration of phosphate ions (found by Eble and Pfeiffer (1935) to be 0.19 millimol. per 1000 g. H_2O) is of the same order as the calculated values, but in view of the relatively high solubility of $MgCO_3$ the Mg content of the white is surprisingly low.

An extension of Buckner, Martin and Peter's (1925) experiment showed that these differences are not due to the impermeability of the membranes separating shell and white. After removal of white and yolk, CO_2 was bubbled through 15 c.c. 0.05 M. $NaHCO_3$ in contact with intact membrane for 4 hr. at 25° C. The Ca concentration was then 1.70×10^{-3} M (estimated gasometrically by the method of van Slyke and Sendroy (1929)). When the membranes were removed and the experiment repeated the corresponding concentration was $1·75 \times 10^{-3}$ M.

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† Solubility of $CaCO_3$ at 25° C (Frear and Johnston 1929), solubility of $MgCO_3$ at 25° C (Kline 1929), solubility of $Ca_3(PO_4)_2$ at 38° C (Sendroy and Hastings 1926–7).

‡ The solubility of $CaCO_3$ decreases with increasing temperature.

Other changes accompanied the reaction

$$CaCO_3 + H_2CO_3 \rightarrow Ca^{++} + 2HCO_3^- \qquad (13)$$

When coarsely powdered shell was tipped from the side bulb of a Warburg manometric vessel into egg-white or 0·05 M $NaHCO_3$ in equilibrium with 0·96 atm CO_2 there was an uptake of CO_2 and from separate experiments, an increase in HCO_3^- concentration. The final uptake increased with the weight of shell used and could be made many times greater than that due to reaction (13).* The cations dissolving from the shell were not identified but the solution of roughly one-half the reported $MgCO_3$ content of the shell would account for the results, the remainder of the $MgCO_3$ may not have been accessible.

In the intact egg the solution of carbonates from the shell is probably very slow. It is also possible that the layer of shell immediately adjoining the membrane contains no solid $MgCO_3$ and that the remainder is not accessible to the white.

SUMMARY

The combined CO_2 of egg-white in equilibrium with different partial pressures of CO_2 was measured over the range $p = 0·05$-$1·0$ atm CO_2 at 25° C.

It was assumed that the combined CO_2 was present in the form of bicarbonate. On this basis the buffer value per g. protein was $4·8 \times 10^{-5}$ for the pH range 6·6-7·8.

The reasonable values calculated for the pH of the white and the buffer value of the proteins supports the assumption that the amount of carbamino CO_2 present is small.

The value of the Bunsen solubility coefficient for CO_2 and white at 25° C was 0·71. This value is about 8 % greater than the value calculated from the salt and water content of the white. It is possible that, as in blood serum, the excess solubility is caused by the presence of traces of lipoids.

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* Using pure calcite in the side bulb, 0·05M $NaHCO_3$ and $p = 0·96$ atm CO_2, the final increase in HCO_3 concentration was 0·0028 mol./l. The value was independent of the weight of calcite or the volume of liquid and agrees with the value calculated from the solubility data of Frear and Johnston (1929), namely 0·0025 mol./l. Using 0·1 g. shell, 1 c.c. 0·05M $NaHCO_3$ and $p = 0·96$ atm CO_2, the final increase in HCO_3^- concentration was 0·013 mol./l.

The uptake of CO_2 by shell and yolk was measured

The buffer value of egg-white and the relation between the salts of shell and white have been discussed

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On the corpora allata of dipterous insects II

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(*Communicated by A D Imms, F R S—Received* 27 *June* 1938)

In an earlier paper (Burtt 1937) it was suggested, on morphological and histological evidence, that Weismann's ring in the larva of *Calliphora* represents the modified corpora allata Fraenkel (1935) had previously found that pupation in *Calliphora* is induced by a hormone which is secreted in the region of the central nervous system Accordingly, I suggested that Weismann's ring was the organ responsible for this effect Simultaneously with the appearance of my paper, Hadorn (1937*a*) published experimental evidence on the function of Weismann's ring ("ring gland") in the larva of *Drosophila melanogaster*, and came independently to the same conclusion as myself regarding the significance of the ring Hadorn used the technique of Ephrussi and Beadle for transplantation in *Drosophila* larvae In his first paper, Hadorn (1937*a*) describes the results of experiments on the mutant "lethal giant"—a form in which pupation is either greatly delayed or absent, and the larva reaches a size exceeding the normal Hadorn found that pupation in "lethal giant" larvae could be markedly accelerated if a Weismann's ring were implanted from a normal larva which was just about to pupate On the other hand no accelerating effect was produced by implanting tissues other than the ring In a later note Hadorn (1937*b*) states that a similar acceleration of pupation is obtained in normal *Drosophila* larvae by transplanting rings from those ready to pupate into younger larvae Three rings gave a greater accelerating effect than one The ring of *Lucilia* was found to be active when implanted in *Drosophila*—a result similar to the finding of Wigglesworth (1936) in which the blood of a moulting *Rhodnius* induced moulting in *Triatoma* and *Cimex*

The present paper deals with the results obtained by destroying Weismann's ring in the larva of *Calliphora* and thus approaches the problem from the opposite point of view from Hadorn

MATERIAL AND METHODS

Larvae of *Calliphora vomitoria* L were used throughout the experiments

It will be seen from fig 1 that Weismann's ring (*w*) is traversed by tracheae These tracheae have a very distinctive shape which is easily visible through the transparent skin of the larva, and they thus afford an exact guide to the position of the tissue of the ring The destruction of the ring was accomplished in one of two ways The first method was to employ an electrically heated wire cautery A fine point was made by putting a sharp bend in a piece of wire 0 1 mm in diameter, the free ends were soldered to copper leads and these in turn were connected to an accumulator with a variable resistance in series The temperature of the wire could be exactly adjusted to that needed to cauterize a piece of tissue without causing undue burning (about 150° C) The wire never became hot enough to cause oxidation so that platinum was unnecessary and constantan was quite satisfactory

The procedure was as follows Larvae were anaesthetized with ether and placed on the stage of the binocular microscope They were flattened slightly at the level of the nervous system, by pressure with the forceps, until Weismann's ring came into view, lying closely pressed against the dorsal body wall The hot wire point was then pressed all round the ring and the tissue cauterized

The second method consisted in the direct removal of Weismann's ring The larva was anaesthetized with ether and placed dorsal side uppermost in a wax dish containing 0 9 % sodium chloride solution A circular wire loop was pressed on to the anterior end in such a way that the central nervous system, and the tissue lying above it, tended to bulge into the area enclosed by the loop A cut was then made with a fine pair of scissors in the dorsal body wall immediately above Weismann's ring, a fine hook was now inserted, the ring was drawn out and freed from its attachments by cutting with the scissors Once this had been accomplished, any projecting tissue was gently pushed back and the cut closed, if necessary, with celloidin solution

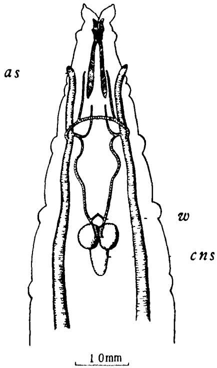

FIG 1 *Calliphora vomitoria* L Tracheae supplying Weismann's ring Lettering *as* anterior spiracle *cns* central nervous system, *w*, tracheae which indicate the position of Weismann's ring

Of these two methods, the first was more rapidly carried out, but the second had the advantage of directness in that one could examine the tissue which had been removed and thus ascertain whether the operation had been complete Moreover, it was essential ultimately to dissect the operated larvae, and examine them for Weismann's ring The second method was superior in this respect, because the examination was not complicated by the presence of cauterized tissue

As stated before (Burtt 1937), the ring is composed of large cells with large nuclei These have a characteristic appearance in whole preparations stained with borax carmine Accordingly this method of staining was used in examining larvae with a view to finding whether any of Weismann's ring was still remaining

It must be pointed out that removal of Weismann's ring also involves removal of the median sympathetic ganglion underneath it (see fig 2, *m*) together with a piece of aorta and tracheal tissue

The second technique for removing Weismann's ring was in part suggested by the method employed by Aschner and Ries (1933) for removing the mycetome in *Pediculus*

DESTRUCTION OF WEISMANN'S RING IN MATURE LARVAE

The following experiments show the effect of destroying Weismann's ring in larvae which have ceased to feed, and are in the resting (Lowne 1890 2) or prepupal condition (Wardle 1930)

A group of larvae was used which were as far as possible at the same stage of development, viz 2 3 days before pupation Of these twenty-nine had the ring removed by the second method, they constitute Group 1 in Table I Another thirty larvae were used as controls, they were anaesthetized and a cut was made in the region over Weismann's ring but the ring was not touched (Group 2) A further twenty-six larvae were used for operations on tissues adjacent to the ring, in twelve of these the tracheae were cut anterior to the ring (Group 3), and in fourteen a piece of the imaginal bud of the eye was removed from the left side (Group 4) All larvae were allowed to recover from the anaesthetic and were then placed on moist filter paper in Petri dishes at 25° C The results are shown in Table I It will be seen that pupation did not occur in the surviving larvae of Group 1 where the ring had been removed On the other hand, pupation occurred in all the surviving control larvae with the exception of one specimen in Group 3 Fourteen days from the beginning of the experiment sixteen of the larvae of Group 1 were still capable of movement During the next 7 days these died without pupating

We may conclude that removal of Weismann's ring prevents pupation The nature of the controls makes it very unlikely that this effect is due to injury to tissues other than the ring

The same results are obtained by the use of the first (cautery) method In an experiment fifty-two larvae were used of the same age as before (2-3 days before pupation) The ring was cauterized in twenty-six of them In the twenty-six controls a small area was cauterized on the same segment as Weismann's ring but care was taken to avoid that organ All the controls pupated within 4 days from the beginning of the experi-

TABLE I RESULTS OF EXPERIMENTS INVOLVING REMOVAL OR CAUTERIZATION OF WEISMANN'S RING IN MATURE LARVAE OF *Calliphora*

Treatment	Number of larvae which died (*) or pupated on each of the days shown after treatment														Total
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	
(1) Ring removed	9*	—	1*	—	—	1*	—	—	—	—	2*	—	—	—	13 died
from 29 larvae	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0 pupated
															16 unpupated
(2) Controls,	—	—	1*	—	—	—	—	—	—	—	—	—	—	—	1 died
30 larvae	—	12	10	4	3	—	—	—	—	—	—	—	—	—	29 pupated
															0 unpupated
(3) Tracheae cut,	—	2*	—	—	—	—	—	—	—	—	—	—	—	—	2 died
12 larvae	—	—	—	5	1	2	1	—	—	—	—	—	—	—	9 pupated
															1 unpupated
(4) Eye bud injured,	—	—	3*	—	—	—	—	—	—	—	—	—	—	—	3 died
14 larvae	—	—	1	5	4	—	1	—	—	—	—	—	—	—	11 pupated
															0 unpupated
(5) Ring cauterized,	—	—	1*	—	—	—	—	1*	—	—	1*	—	—	—	3 died
26 larvae	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0 pupated
															23 unpupated
(6) Controls,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0 died
26 larvae	—	4	20	2	—	—	—	—	—	—	—	—	—	—	26 pupated
															0 unpupated

ment None of the larvae in which the ring had been cauterized ever pupated Fourteen days after the beginning of the experiment three of these larvae had died but twenty three were alive and capable of movement The prepupal larvae (unlike the younger larvae) are very insensitive to the effects of the cautery and the method thus offers a simple way of preventing pupation in *Calliphora*

THE EFFECT OF SEVERING THE NERVE CONNEXIONS OF WEISMANN'S RING

The results of the preceding section are somewhat complicated by the fact that cutting the nerves to Weismann s ring has much the same effect in preventing pupation as removal of the ring itself

The ring is in intimate contact with the median sympathetic ganglion lying immediately beneath it (see fig 2 *m*) This ganglion is supplied with nerves (fig 2 *n*) from the cerebral commissure and joined by a short branch (fig 2, *r*) from the recurrent nerve As before in operating the tracheae traversing the ring were used as a guide to its exact position A fine knife was made by grinding the point of a needle so that one side of the point formed an edge about 0 3 mm long Larvae were anaesthetized and held with the forceps whilst the knife was inserted laterally through the body wall until it came to lie under the tracheae at the point where they pass into the brain In fig 2 we must imagine the knife to lie at right angles to the plane of the paper with its edge between the cerebral commissure and the nerves to the median sympathetic ganglion Once in this position the knife could be drawn backwards until the tracheae and the nerve connexions were cut

Some fifty seven larvae were taken of an age about 4 days before pupation The above operation was attempted in thirty of these and in the twenty-seven controls the tracheae were cut in front of the ring The results are shown in Table II in the first case nineteen larvae and in the controls only one larva failed to pupate Dissections were subsequently made of the operated larvae They were opened by a longitudinal cut passing to one side of the middle line and the internal organs were immediately washed with Bouin's fluid Under these circumstances the sympathetic system could be easily traced Considering those which pupated in the group of thirty with the ring cut in three of the six pupae the operation had failed and the nerves were intact, whilst in the other three the cut had passed across the ring so that half of it still retained its nerve connexions Now let us turn to the nineteen larvae which had failed to pupate In twelve of these the only nerves severed were those joining the ring to the brain and to the sympathetic system, whilst the aorta and the recurrent nerve were apparently intact In the remaining seven of the nineteen one or both of these last were in addition cut across No abnormality was detected in the control larva which had failed to pupate

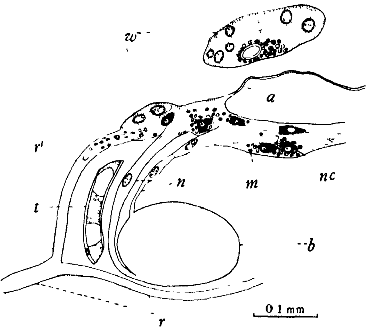

Fig 2 *Calliphora vomitoria* L Median sagittal section between the cerebral hemispheres of a mature larva Lettering *a*, aorta, *b* brain, *m*, median sympathetic ganglion, *n*, nerves to median ganglion *nc*, nerve cells, *r* recurrent nerve, *r′*, branch of recurrent to Weismann's ring, *t*, trachea, *w*, Weismann's ring

The ring was, of course, present in all the nineteen larvae where it had been severed Sections were made in two of them, the cells appeared rather shrunken but perfectly recognizable

The question naturally arises whether cutting the recurrent nerve at a level posterior to Weismann's ring would have any influence on pupation This operation cannot be performed with much certainty, but in thirty-three larvae a cut was made posterior to the ring Of these, eight died within a day of the operation and twenty-five pupated Examination showed that in nine of these pupae the recurrent nerve was severed in the region posterior to the ring

We may conclude from the above experiments that the function of Weismann's ring is inhibited if its nerve connexions are cut Wigglesworth

TABLE II EFFECTS OF CUTTING THE NERVE CONNEXIONS TO WEISMANN'S RING IN MATURE LARVAE OF *Calliphora*

Treatment	Number of larvae which died (*) or pupated on each of the days shown after treatment														Total
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	
Nerves to ring cut, 30 larvae	—	2*	3*	—	—	—	—	—	—	—	—	—	—	—	5 died
	—	—	1	1	2	—	2	—	—	—	—	—	—	—	6 pupated
															19 unpupated
Controls, 27 larvae	—	2*	4*	—	2*	—	—	—	—	—	—	—	—	—	8 died
	—	—	5	5	5	3	—	—	—	—	—	—	—	—	18 pupated
															1 unpupated

(1934) found that moulting could be prevented in *Rhodnius* if the nerves between the brain and the corpus allatum were cut, and Pflugfelder (1937) obtained the same effects in *Dixippus* by cutting the nerves to the corpus allatum as by removal of the gland

DESTRUCTION OF WEISMANN'S RING IN YOUNGER LARVAE

We must now consider the possible function of Weismann's ring in larvae which are still feeding Up to the time of writing the operation has not been successfully performed on the first and second instar larvae What follows, therefore, applies only to the third (and last) larval instar The destruction of Weismann's ring at this stage was effected by each of the two methods The feeding larvae were particularly susceptible to the cautery, and only 10–20% continued to feed and move actively after the operation The death rate was lower when the ring was removed by the second method, and as both techniques gave the same results, only the larvae operated on by the latter method need be considered

Weismann's ring was removed by the second method in thirty-four larvae of a length of 12–13 mm (age about 72 hr at 25° C) Of these, nineteen died as a result of the operation and fifteen continued to feed and crawl about in a normal way They were supplied with beef and kept at 25° C The lives of these larvae were followed, and a comparison with controls in which a simple cut had been made showed the following effects (1) the larvae do not pupate, (2) they continue to feed for a period greatly exceeding the normal In these circumstances the crop remained full and one could be sure that they were still feeding by putting the larvae on beef mixed with carmine, when the red colour became visible throughout the alimentary canal Three examples will make this clear Larva (1) was operated on 11 March 1938 and continued to feed until 19 April 1938 when it became sluggish and was killed on 21 April 1938 This larva showed a prolongation of the period of feeding amounting to 39 days which may be compared with the normal total of 4 days Larva (2) was operated on 24 February 1938 and killed and dissected on 25 March 1938 The period of feeding in this case lasted 28 days Larva (3) was operated on 24 February 1938 and examined on 15 March 1938—the prolongation of the feeding period in this instance was 19 days

It will be seen from these results that, although the larval life has been much prolonged, the development never reached the stage at which feeding ceased

As regards the structure of the operated larvae their body size was slightly less than that of the normal larvae at the end of feeding A better understanding of the stage reached is obtained from an examination of the imaginal buds The bud selected for measurement was that which gives rise to the fore wing and neighbouring mesothoracic structures, as was shown by Weismann (1864) Because of its large size and plate-like shape, it makes a very convenient object for careful measurements The following considerations apply equally well to the other imaginal buds (e g the ventral thoracic) In any given larva the wing bud of one side was stained and mounted whole whilst that of the other side was sectioned In this way an idea could be gained both of the superficial area of the bud and its thickness

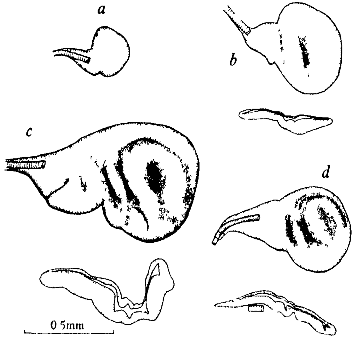

Fig 3 Stages in the growth of the wing bud in C *vomitoria* L during the third larval instar (*a*) normal 12 mm larva (*b*) full grown larva, at the time of cessation of feeding (*c*) 48 hr later than (*b*) (*d*) wing bud from larva in which Weismann's ring has been removed at a stage corresponding to (*a*) above Longitudinal sections of the buds are shown below (*b*), (*c*), and (*d*)

Consider first the development of the normal wing bud, three stages of which are shown in fig 3, (*a*) is from a normal 12 mm larva of the stage corresponding to that at which Weismann's ring was removed, (*b*) a larva which has just ceased to feed, and (*c*) a larva 48 hr older, during the prepupal period It is clear that a marked increase both in area and thickness occurs during the prepupal period Further, such increase in size of the bud takes place at a time prior to the onset of puparium formation (the critical period of Fraenkel 1935) This was readily checked by destroying Weismann's ring and keeping the larva for 36 hr before dissection of the imaginal buds Under these conditions if the larva in question had already entered the critical period, then pupation would have occurred

If we turn to the larva with Weismann's ring removed (fig 3, *d*) comparison with (*a*) of the controls makes it clear that the wing bud has undergone further growth, but that it has been arrested at a stage distinctly smaller than the control specimen (*c*) It is about equivalent to the normal larva at the cessation of feeding i e fig 3 (*b*) With slight variations the same arrest of the growth of the imaginal buds was found in the other larvae in which Weismann's ring had been removed at the 12 mm stage

It follows from the above that the ring exercises an influence on the development of the imaginal buds before the time at which it induces pupation

The only other internal organs in the arrested larva which have been found to show any abnormality are the Malpighian tubes which may be distended with calcium carbonate to a degree above the normal The gonads it may be observed are not larger than in the controls at the time of pupation

DISCUSSION

It is natural to compare the results obtained from *Calliphora* with the findings of Hadorn in *Drosophila* The close similarity in structure and development of the two insects makes one expect that the role of Weismann's ring would be the same in each case Bodenstein (1938) has performed ligaturing experiments on *Drosophila* larvae and obtained essentially the same results as Fraenkel (1935) working on *Calliphora* Further, the drawings given by Auerbach (1936) of the development of the wing bud in *Drosophila* show a series very similar to that given above for *Calliphora* Hadorn (1937*b*) states however that the implanted ring only hastens the formation of the pupa and does not affect the development of the imaginal buds for if pupation is made to take place too early, then the pupa does not develop adult characters This rather suggests that the influence of Weismann's ring on pupation and on the growth of the imaginal buds are separable effects Alternatively, it may be that the functions of the organ are different even in insects so apparently similar as *Calliphora* and *Drosophila*

In this connexion it is interesting to consider the effects of removal of the corpora allata in insects of other orders In Orthoptera, Weed (1936) has given preliminary results on the grasshopper *Melanoplus differentialis* She finds that loss of the corpora allata in the last instar but one of the life cycle does not prevent the occurrence of the final moult On the other hand, it does prevent the adult from becoming sexually mature This last finding is in agreement with the results of Wigglesworth (1936) which indicate that the corpora allata are responsible for the onset of sexual maturity in *Rhodnius* Pflugfelder (1937) removed the gland from *Dixippus morosus* (Orthoptera) as early as the third instar The operated nymphs still underwent two ecdyses but started to lay eggs in the 5th instar instead of in the 7th as normally Obviously in this case the corpora allata are not necessary for reproduction and moulting At the same time, they exercise one function similar to that in *Rhodnius* for they control the rate at which the insect becomes adult Wigglesworth (1936) obtained precocious adults in place of nymphs by removing the head during the critical period—an effect to be ascribed to a limitation of the period during which the corpus allatum can discharge its secretion into the blood Another point raised by the work of Weed and Pflugfelder is in regard to the onset of sexual maturity here even within the limits of one order there appears to be a difference in the control exerted by the corpora allata

The work of Bounhiol (1936, 1937*a* 1937*b*) has perhaps more bearing on the present paper because it has been carried out on a holometabolous insect, viz the silk worm *Bombyx mori* Bounhiol removed the corpora allata from larvae of ages varying from 1 to 14 days after the last larval moult Pupae were obtained no matter at what stage the operation was performed He also finds (Bounhiol 1937*a* *b*) that in the preceding instar loss of the corpora allata may alter the development to the extent of causing pupation to take the place of the normally ensuing larval ecdysis—once again an instance of precocious maturity following removal of the corpora allata Kuhn and Piepho (1936) using the moth *Ephestia kuhniella* and Plagge (1938) working on the larvae of the hawk moth *Deilephila euphorbiae* agree with Bounhiol that the corpora allata are not to be regarded as the source of the pupating hormone in Lepidoptera Both authors find that removal of the supra oesophageal ganglion (brain) prevents metamorphosis (following Kopec 1922) Further, they can induce pupation in such larvae by grafting another brain into their bodies, and claim that pupation can be made to occur by this means even in cases where the corpora allata are not present in the graft

The results obtained in the Lepidoptera appear to suggest that the functions of the corpora allata in that order are different from those of Weismann's ring in the Diptera But a possible complication must not be overlooked, and here the work of De Lerma (1937) is relevant to the discussion He has made a histological study of the corpora allata and corpora pharangea in the Orthoptera The corpora pharangea are a pair of ganglion like structures variously termed by other authors the corpora cardiaca, oesophageal ganglia or occipital ganglia De Lerma confirms the previous view that the corpora allata are composed of cells of non-nervous type, but he also concludes that the corpora pharangea contain a large proportion of non nervous tissue He goes on to point out that a similar condition is found in Coleoptera (*Hydrophilus*) and in Hymenoptera (*Vespa*) It may be added that Pflugfelder (1937), in his account of the histology of the corpora cardiaca (= corpora pharangea) of *Dixippus*, finds a large number of cells of non-nervous type De Lerma further claims that these cells in the corpora pharangea give signs of secretory activity in that they show an accumulation of granules and drops in the cytoplasm In a recent paper Pflugfelder (1938) brings additional observations on this point He shows that in termites the hypertrophy of the corpora allata which takes place in the mature sexual forms is accompanied by a corresponding increase in the size of the corpora cardiaca (= corpora pharangea) There is no experimental evidence as to what the function of the corpora cardiaca might be At the same time one must not overlook the possibility that the sympathetic system of insects contains more than one type of endocrine organ

In view of the marked variations in the arrangement of the corpora allata and cardiaca in different insects, it may be that, in operations aimed at removing the corpora allata, there have been differences in the degree to which the corpora cardiaca have been injured We saw that in *Calliphora*, where the sympathetic system is much approximated, removal of Weismann's ring involves removal of the adjacent sympathetic ganglion In forms such as *Dixippus*, *Melanoplus* and *Bombyx* a sharper separation of the corpora allata and the corpora cardiaca may be possible

Alternatively, it may be that the corpora allata exhibit marked differences of function in different orders, and perhaps even in different members of the same order The work on various species of insects is obviously not yet far enough advanced to allow one to discriminate between these possibilities

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SUMMARY

Evidence is given concerning the function of Weismann's ring which, as was suggested by the author in 1937, represents in *Calliphora* the corpora allata of other insects Methods are described for extirpating Weismann's ring in the larva of *Calliphora* Pupation can be prevented either by removing Weismann's ring or by cutting the nerve connexions to this organ Removal of the ring in larvae which are still feeding causes an arrest of development Such larvae continue to feed for a period greatly exceeding the normal, and the growth of their imaginal buds is arrested It is concluded that Weismann's ring secretes the pupating hormone discovered by Fraenkel, and in addition influences processes taking place prior to pupation The work of Hadorn on Weismann's ring in *Drosophila* is briefly reviewed, and the somewhat discordant results obtained by workers who have removed the corpora allata in species of Orthoptera and Lepidoptera are discussed

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The effect of androgenic substances on the growth of the teat and mammary gland in the immature male guinea-pig

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(Plate 10)

It is commonly believed that in most species of mammals the mammary gland in the male remains rudimentary throughout life and consists only of a duct system at the base of the teat which itself remains smaller than in the pubertal female. Nevertheless, there is no doubt whatever that the male mammary gland is equipotential with that of the female as regards its capability of responding to the growth stimulus of ovarian hormones and consequently the male has been extensively used in studies of the experimental development of the mammary gland (see numerous citations by Nelson 1936).

The above generalizations are not equally true for all species however, since there is a certain amount of evidence that in some mammals, notably the rat and man, the male mammary gland undergoes at puberty more rapid and extensive growth than that required merely to keep pace with general body growth. The occurrence of moderate mammary development at some stage in the life cycle in the male rat may be inferred from the observations of Turner and Schultze (1931) who mention the presence of lobules in the mammary gland of the mature male while McEuen, Selye and Collip (1936) actually observed considerable growth of the male mammary gland in the rat at puberty.

In man, the observations of Andrews and Kampmeier (1927) suggest that the male mammary gland normally undergoes some considerable degree of development, while Jung and Shafton (1935) claim to have observed enlargement of the mammary gland detectable by palpation in boys at puberty.

The results of McEuen, Selye and Collip (1936) suggest that the development of the mammary gland in the male may be due to the endocrine activity of the testes, since the growth which occurs at puberty was prevented by prepubertal castration, while mammary involution followed castration of mature males On the other hand, Turner and Schultze (1931) reported that castration caused no regression of the mammary gland of the male rat, while Smelser (1933) reported that the mammary apparatus (teat and mammary duct system) of the male guinea pig undergoes no regression after castration

In addition to these species for which there is evidence that the male mammary gland normally exhibits a moderate degree of growth and development during the life cycle abnormal mammary development (gynaecomastia) in the male, sometimes accompanied by milk secretion, occasionally occurs in various species Seifert (1920) cites reports of various cases of gynaecomastia in man Folley, Guthkelch and Zuckerman (1938) have observed one case of abnormally extensive mammary development in a male rhesus monkey Cases of abnormal development of the male teat and mammary gland sometimes accompanied by milk secretion appear to occur with greater frequency in the goat than in other animals (see Herre 1933 and Spann 1933) though according to Turner and Gomez (1936) mammary development in the male goat is normally very restricted

Growth of the male mammary gland at puberty might be accounted for in two ways (*a*) production of a primarily oestrogenic substance such as evidently occurs in many male mammals, (*b*) action of the testicular hormone itself In support of the latter hypothesis may be cited the fact that administration of testosterone to rats has been shown to cause mammary development (Selye McEuen and Collip 1936 Astwood Geschickter and Rausch 1937) The latter workers record that the mammary gland of the mature male rat differs from that of the pubertal female in having a relatively small area but a dense lobule-alveolar system and that administration of testosterone induces development of this type in both male and female immature gonadectomized rats Further Nelson and Gallagher (1936) reported that administration of the artificially produced androgens androstandiol and androstendione to rats caused proliferation of the mammary gland, while androsterone was inactive in the dose used

It is likely therefore that the normal growth of the male mammary gland is due to the gynaecogenic action of the male hormones produced by the testes and there appears to be no need to postulate the action of the oestrogenic substance produced by the male body The causation of gynaecomastia further discussed below may be more complicated

Recent investigations (e g Butenandt and Kudszus 1935, Deanesly and Parkes 1936, Korenchevsky and Hall 1937) have shown that many androgenic substances possess gynaecogenic properties, and since the power to promote development of the mammary apparatus is undoubtedly a gynaecogenic property, it was considered of interest to study the effect of a large series of androgens on the development of the mammary apparatus It was hoped that such a study would yield further information on the nature of the testicular control of the normal male mammary gland and also on the possible causes of gynaecomastia in the male

EXPERIMENTAL

Experimental animals and treatment The work was carried out on normal and castrated immature male guinea pigs weighing about 150–170 g at the beginning of treatment Groups of five or more animals were used but in three instances one member of a group died All animals grew steadily throughout the experiment Castration was performed through a mid-ventral incision under Avertin anaesthesia (0 65 ml $2\frac{1}{2}$ % Avertin per 100 g body weight intraperitoneally) The dose of all androgens was 2 mg daily except for testosterone propionate of which the daily dose was 2 36 mg (= 2 mg testosterone) The total number of injections varied somewhat from group to group, but as will be seen in Table I it was always round about 30 When possible, the daily dose of androgen was dissolved in 0 2 ml sesame oil, but for the relatively insoluble diols 0 8 ml was necessary In order to examine the effect of volume of solution on response, testosterone and its propionate were injected at both dilutions Two control groups received daily injections of 0 2 and 0 8 ml sesame oil respectively

Method of assessing mammary development Three obvious methods are available for studying mammary development, namely, measurements of teat length and examination of mounts of the whole gland or of histological sections Smelser (1933), in his studies of the experimental development of the mammary gland in the male guinea pig, relied chiefly on the first He considered that increases in teat length ran parallel with general mammary development and that alterations in teat length are far easier to measure than changes in mammary gland area He also pointed out that measurement of teat length possesses the further advantage that a series of observations can be made on one animal Since the teat length technique is so convenient, it has been used in the work described in this paper, though in all cases histological examination of tissue surrounding the base of the teat has been made at the conclusion of the experiment

Duplicate measurements of both teats of each animal were made to the nearest 0 1 mm with small screw dividers at 7 day intervals beginning on the first day of each experiment, body weight being recorded at the same time The measurements for all animals in a group on a given day were averaged so that each point on the teat growth rate curves represents the mean of 16–36 measurements Final measurements were made on the day after the last injection, when the guinea pigs were killed with coal gas The mammary duct system and surrounding connective tissue, and the teat, were dissected off and fixed in Helly's modification of Zenker's fluid Sections were cut at 8μ and stained with haematoxylin and eosin

TABLE I

Group no	Injections	No of guinea pigs in group	No of daily injections
	(*a*) 2 mg androgen dissolved in 0 2 ml sesame oil daily		
1	Sesame oil	8	31
2	Sesame oil	4(castrated)	31
3	Δ_4 Androstendione	5	32
4	*cis* Androsterone	9	31
5	Δ_5 *trans* Dehydroandrosterone	5	31
6	Dihydrotestosterone*	5	32
7	17-Methyltestosterone	5	34
8	Testosterone	4	32
9	Testosterone propionate	5	32
10	Testosterone propionate	4(castrated)	32
	(*b*) 2 mg androgen dissolved in 0 8 ml sesame oil daily		
11	Sesame oil	9	34
12	*cis* Androstandiol	5	28
13	Δ_5 *trans* Androstendiol	5	28
14	Δ_5 *trans* Androstendiol	5(castrated)	28
15	Testosterone	5	27
16	Testosterone propionate	5	27

* 3 keto aetio *allo*cholanol-17

The rate of teat growth in the normal immature male Previous observations (Bottomley and Folley 1937) had suggested that in the young male guinea-pig the rate of teat growth relative to the rate of general body growth is approximately isometric (see Huxley and Teissier 1936) over a period of 62 days during which the body weight increases from about 230 to 430 g This, in effect, means that the increase in teat length keeps pace with the increase in body length Many growth phenomena obey the simple allometric law of Huxley (1932) which is usually written $y=bx^{\alpha}$, where y = the length of the organ under consideration, x = the length of a reference organ, and b and α are constants This relation may also be expressed in the form

$$\log y = \log b + \alpha \log x,$$

so that if growth data obeying this law are plotted logarithmically, a straight line of slope α will be obtained In the case of isometric growth which may be considered as a special case of allometric growth, the second expression becomes $\log y = \log b + \log x$ and the straight line obtained by logarithmic plotting of the data will have a slope equal to unity

The obvious standard for body size with which to compare the teat length (L) is, of course, the body length Owing to the difficulty of obtaining reliable measurements of body length in animals such as the guinea-pig, the one-third power of the body weight ($\sqrt[3]{W}$) was adopted, since it is probable that the rate of increase in this function is approximately proportional to the rate of increase in the body length

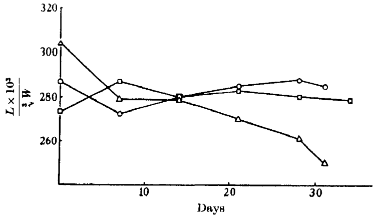

FIG 1 Changes in the ratio $L/\sqrt[3]{W}$ with time, where L = teat length in mm and W = body weight in g ○——○ Guinea pigs receiving daily injections of 0 2 ml sesame oil □——□ Guinea pigs receiving daily injections of 0 8 ml sesame oil △——△ Guinea pigs castrated one week before the first measurement, thereafter receiving daily injections of 0 2 ml sesame oil

Mean values for the ratio $L/\sqrt[3]{W}$ for the two normal control groups (Groups 1 and 11) and the group of castrated controls (Group 2) are plotted against time in fig 1 Such curves are hereinafter referred to as curves of relative teat growth rate

The curves for intact males show that the ratio $L/\sqrt[3]{W}$ remains practically constant, which means that over the time covered by these experiments, teat growth in the young guinea pig is isometric In each curve the point which deviates most from constancy is the first, corresponding to the smallest teat length and the greatest error in measurement

The isometry of teat growth in the intact young male is shown in another way in fig 2 in which the data are plotted logarithmically The twelve points obviously indicate a linear relationship between the logarithm of mean teat length and the mean value of (body weight)$^{\frac{1}{3}}$ and the best straight line fitted by the method of least squares is also shown in fig 2 The slope of this straight line is 1 078, which is sufficiently near to unity to indicate isometric growth For comparison with other data this line is shown as a dotted line in figs 6 7, 8 and 12

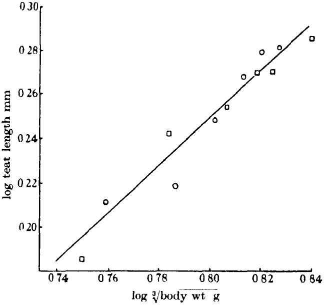

Fig 2 Curve showing isometry of teat growth in the normal male guinea pig The straight line shown in the figure has been fitted by the method of least squares Its slope = 1 078 For isometry the slope should be 1 000 ○ Guinea pigs receiving daily injections of 0 2 ml sesame oil ☐ Guinea pigs receiving daily injections of 0 8 ml sesame oil

The effect of castration on the rate of teat growth In fig 1 the curve relating to the castrated controls (Group 2) shows a decreasing ratio $L/\sqrt[3]{W}$, which means that castration is followed by a phase of negative allometry (see Huxley and Teissier 1936) There is no absolute decrease in the length of the teats, but they merely cease to increase in length after castration

This result, which is not in accord with those of Smelser (1933), suggests that the isometric growth of the teat in the immature male is maintained by the endocrine activity of the testes

The effect of unsaturated androgens on teat growth The mean relative rates of teat growth for the groups of guinea-pigs receiving injections of

Δ_5-*trans* androstendiol, testosterone propionate (lower dilution), Δ_5-*trans*-dehydroandrosterone and Δ_4-androstendione, are plotted in fig 4 For the sake of clarity all the curves in fig 4 (and in figs 5, 9, 10 and 11) were made to originate from the same point by plotting the *increase* in the ratio $L/\sqrt[3]{W}$ along the vertical axis against time along the horizontal axis

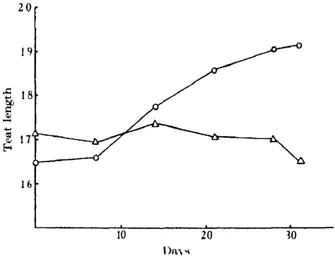

FIG 3 The effect of castration on teat length in the young male guinea pig △— △ Guinea pigs castrated one week before the 1st measurement thereafter receiving daily injections of 0 2 ml sesame oil ○ - ○ Guinea pigs receiving daily injections of 0 2 ml sesame oil

All of these androgens except Δ_4 androstendione caused more rapid relative teat growth than that shown by the controls (i e positive allometric growth), Δ_5-*trans* androstendiol giving by far the greatest effect The teats of the animals receiving injections of Δ_4 androstendione grew at about the same relative rate as those of the controls (i e approximately isometrically) In a preliminary communication (Bottomley and Folley 1938) it was reported that in these experiments treatment with all of the androgens listed in Table I except Δ_5-*trans* dehydroandrosterone, caused testicular atrophy, presumably by depression of the gonadotropic potency of the hypophysis Since castration is followed by cessation of teat growth, it follows that most of the curves shown in fig 4 (and also figs 5, 9, 10 and 11) express the resultant of two tendencies, namely the tendency of the teat to exhibit positive allometry under the stimulus of the administered androgen and a slight tendency to exhibit negative allometry because of the "castration effect" From the results of Bottomley and Folley (1938) the magnitude of the latter may be assumed to be practically the same for all the androgens used except Δ_5-*trans*-dehydroandrosterone which had no action on the testis in the dosage given and *cis*-androsterone which was less active than the other androgens It follows that the teat growth-promoting activities of Δ_5-*trans*-androstendiol and of testosterone

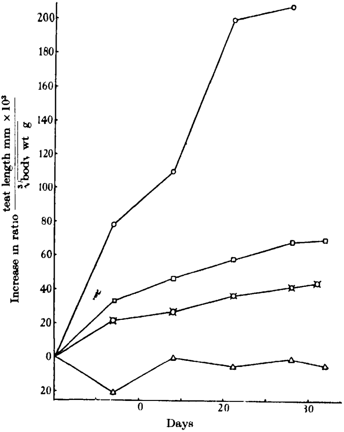

FIG 4 Effect of unsaturated androgens on relative rate of teat growth ○——○ Guinea pigs receiving daily injections of Δ_5 *trans* androstendiol □——□ Guinea pigs receiving daily injections of Testosterone propionate (in 0 2 ml sesame oil) ¤——¤ Guinea pigs receiving daily injections of Δ_5 *trans* dehydroandrosterone △——△ Guinea pigs receiving daily injections of Δ_4 androstendione

propionate relative to that of Δ_5-*trans*-dehydroandrosterone is a little greater than indicated by fig 4, and that Δ_4-androstendione must be slightly active since it maintained isometric teat growth despite the "castration effect"

Fig 5 shows similar curves for the groups of guinea-pigs given testosterone (at the lower dilution) and 17-methyltestosterone In both cases

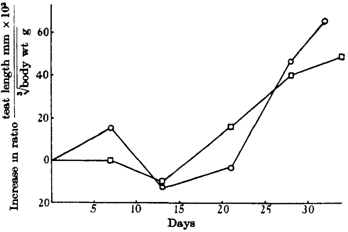

FIG 5 Effect of unsaturated androgens on relative rate of teat growth ○——○ Guinea pigs receiving daily injections of 2 mg testosterone in 0 2 ml sesame oil □——□ Guinea pigs receiving daily injections of 2 mg 17 methyltestosterone in 0 2 ml sesame oil

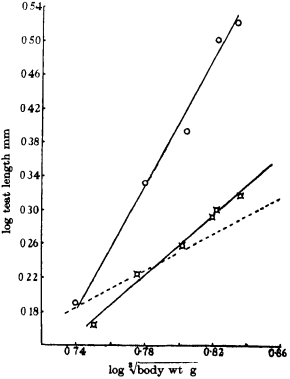

FIG 6 Effect of unsaturated androgens on the relative rate of teat growth ○ Guinea pigs receiving daily injections of Δ_5 *trans* androstendiol ☒ Guinea pigs receiving daily injections of Δ_5 *trans* dehydroandrosterone

there was an initial period during which the teat exhibited approximately isometric growth followed by a phase of positive allometry The initial lag in the relative teat growth rate in the experiments with testosterone (lower dilution) and 17-methyltestosterone may be due to the "castration effect" setting in with especial rapidity and, for a time, counteracting the direct effect of the androgens on the teat

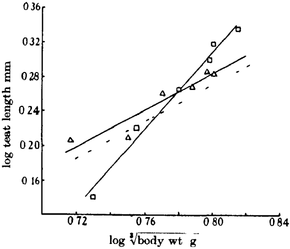

FIG 7 Effect of unsaturated androgens on relative rate of teat growth □ Guinea pigs receiving daily injections of 2 0 mg testosterone as propionate in 0 2 ml sesame oil △ Guinea pigs receiving daily injections of 2 0 mg Δ_4 androstendione in 0 2 ml sesame oil

Logarithmic plotting of the teat growth data shows that Δ_5-*trans*-androstendiol, Δ_5-*trans*-dehydroandrosterone (fig 6) and testosterone propionate at the lower dilution (fig 7) cause growth of the male guinea-pig teat according to the law of simple (positive) allometric growth, each set of points being well fitted by a straight line of slope greater than unity

The straight lines shown in fig 6 and subsequent diagrams (figs 7, 8 and 12) were fitted to their respective sets of points by the method of least squares The line for the animals receiving Δ_4-androstendione is parallel to that for the control animals (i e its slope is approximately unity), a result which again indicates isometric growth of the teat in this group The points for the experiment with 17-methyltestosterone are well fitted by two straight lines (fig 8), the slopes of which indicate that an initial phase of negative allometry is followed by a phase of positive allometry

The results of the experiment with testosterone, in which one animal died, were irregular and it was not possible to fit a line to the points

The effect of castration on the response of the teat to unsaturated androgens Two experiments were carried out in which teat growth under the influence of Δ_5 *trans* androstendiol and testosterone propionate was compared in intact and castrated guinea-pigs The relative growth rates are plotted in fig 9

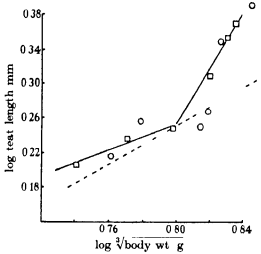

FIG 8 Effect of unsaturated androgens on relative rate of teat growth □ Guinea pigs receiving daily injections of 2 0 mg 17 methyltestosterone in 0 2 ml sesame oil ○ Guinea pigs receiving daily injections of 2 0 mg testosterone in 0 2 ml sesame oil

It will be seen that testosterone propionate is less effective in promoting teat growth after castration This result, which indicates that the testes cause some teat growth, agrees with our other results Δ_5-*trans*-androstendiol, on the other hand, is almost as effective after castration, probably because the power of the testes to promote teat growth is small compared with that of the injected Δ_5-*trans*-androstendiol and its loss comparatively insignificant

The influence of the daily oil volume and of esterification on the capacity of testosterone to promote teat growth Two groups of guinea-pigs received daily injections of 2 mg testosterone as propionate dissolved in 0 2 and 0 8 ml sesame oil respectively Free testosterone was administered to two other groups in the same dilutions The rates of relative teat growth for all four groups are shown in fig 10

Both substances were more active when administered in the smaller volume of oil At the higher dilution esterification had little influence on the effectiveness of testosterone, while at the lower dilution esterification abolished the initial lag in the response to testosterone The result at 32 days, however, was the same for testosterone as for its propionate

The effect of saturated androgens on teat growth The androgens with fully saturated ring structures used in this study, *cis* androsterone, dihydrotestosterone and *cis*-androstandiol, all caused testicular atrophy, but *cis*-androsterone to a much less degree than the others (Bottomley and Folley 1938) The relative rates of teat growth for the groups of guinea-pigs receiving injections of these three substances are shown in fig 11 The corresponding curve for castrated controls is included for comparison

In fig 12 the results are plotted logarithmically in the usual way The points for the group receiving dihydrotestosterone fall very close to a

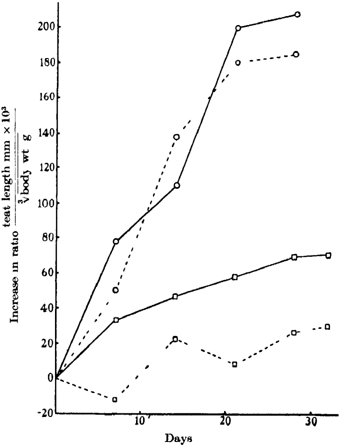

FIG 9 Comparison of the teat growth promoting activities of unsaturated androgens in castrated and intact guinea pigs O——O Intact guinea pigs receiving daily injections of Δ_5 *trans* androstendiol O - - O Castrated guinea pigs receiving daily injections of Δ_5 *trans* androstendiol □——□ Intact guinea pigs receiving daily injections of testosterone propionate (in 0 2 ml sesame oil) □ - - □ Castrated guinea pigs receiving daily injections of testosterone propionate (in 0 2 ml sesame oil)

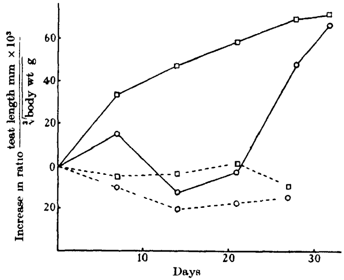

FIG 10 Effect of oil volume and esterification on the teat growth promoting activity of testosterone □——□ Guinea pigs receiving daily injections of 2 0 mg testosterone as propionate in 0 2 ml sesame oil □ □ Guinea pigs receiving daily injections of 2 0 mg testosterone as propionate in 0 8 ml sesame oil ○——○ Guinea pigs receiving daily injections of testosterone in 0 2 ml sesame oil ○ ○ Guinea-pigs receiving daily injections of testosterone in 0 8 ml sesame oil

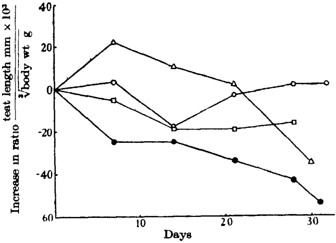

FIG 11 Effect of saturated androgens on the relative rate of teat growth ○——○ Guinea pigs receiving daily injections of dihydrotestosterone △——△ Guinea pigs receiving daily injections of *cis* androstandiol □——□ Guinea pigs receiving daily injections of *cis* androsterone ●——● Guinea pigs castrated one week before the 1st measurement and thereafter receiving daily injections of 0 2 ml sesame oil

straight line parallel to the best fit straight line for the points relating to the control animals Dihydrotestosterone at the high dosage given, therefore, possesses sufficient activity to maintain allometric teat growth in spite of the "castration effect" The points for the androsterone group shown in fig 12 are well fitted by two straight lines the slopes of which would indicate that in this group there was first a phase of negative allometry followed by a phase of positive allometry The results for *cis* androstandiol were so irregular that no attempt could be made to fit a straight line to them In none of these three experiments did the relative rate of teat growth much exceed that for the normal controls nor fall as low as that for the castrated controls Even allowing for the fact that all of these androgens caused testicular atrophy, it may be concluded that their power to promote teat growth is very slight

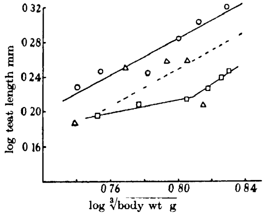

FIG 12 Effect of saturated androgens on relative rate of teat growth ○ Guinea pigs receiving daily injections of dihydrotestosterone □ Guinea pigs receiving daily injections of *cis* androsterone △ Guinea pigs receiving daily injections of *cis* androstandiol

Microscopic examination of the mammary glands Examination of sections from the control glands showed the presence of ducts which in one or two cases were fairly numerous (fig 13, Plate 10)

These observations agree with those of Turner and Gomez (1936) Alveoli were not seen in any control gland and there was little evidence that the duct system was actively proliferating Only in the glands from guinea-pigs treated with Δ_5-*trans*-androstendiol, methyltestosterone and testosterone propionate (lower dilution) was there any clear evidence of stimulation Δ_5-*trans*-androstendiol appeared to be most active of the three compounds as would be expected from the data for teat growth The glands from the guinea-pigs treated with this substance showed fairly extensive duct systems, the lumina of the main ducts being larger than in the controls There were signs of active duct proliferation (budding) and a few alveoli could be seen (fig 14, Plate 10) The sections from the animals receiving testosterone propionate and 17 methyltestosterone showed extensive duct systems which were actively proliferating though in neither case could the presence of alveoli be distinguished The mammary gland of the male guinea-pig, therefore, does not appear to respond to unsaturated androgens by alveolar formation, as does the mammary gland of the rat to testosterone (Astwood, Geschickter and Rausch 1937)

DISCUSSION

Our observations indicate that in the young normal male guinea-pig the growth of the teat just keeps pace with general body growth, but in the castrated guinea-pig there is no growth of the teat The latter result is more in accord with that of McEuen, Selye and Collip (1936) on the male rat than with that of Astwood, Geschickter and Rausch (1937) on the rat and Smelser (1933) on the guinea-pig The present work, as well as that of Selye, McEuen and Collip (1936) and Astwood, Geschickter and Rausch (1937), shows that testosterone will promote growth of the teat and mammary gland and it is unnecessary to assume that mammary development in the mature normal male is due to the action of the oestrogenic substance found in the male body It is conceivable that the abnormal mammary development characteristic of gynaecomastia may be due to the production of abnormally large amounts of oestrogens by the male, but from the present work it might be due to the pathological production by the body under certain conditions of such compounds as Δ_5-*trans*-androstendiol

The unsaturated androgens caused the teat to grow at an accelerated rate relative to the rest of the body, according to the simple allometric law of growth, in spite of the fact that the body weight of the animals increased by about 50 % during the experiments, so that the daily dose of androgen per gram was progressively falling It is likely that the dosage of all the androgens was above the threshold required for maximum growth of the teat

No accurate statement of the relative power of the compounds studied to promote teat growth is possible, since dose response curves were not determined and since the growth phenomena were complicated by "castration effects" of unknown magnitude, but some idea of the relative

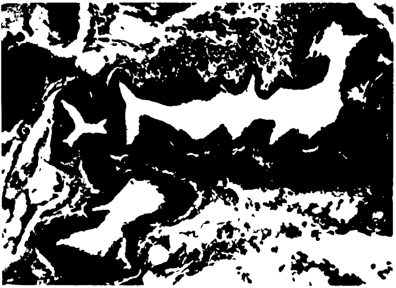

Fig 13 Section of the mammary gland of an immature male guinea pig given daily subcutaneous injections of 0 2 ml sesame oil The duct system shows no signs of proliferation and no alveoli can be seen

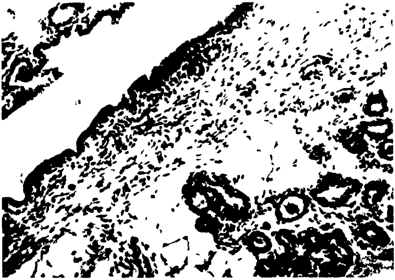

Fig 14 Section of the mammary gland of an immature male guinea pig given 28 daily injections of 2 mg 1, *trans* androstenediol The ducts are wide and show signs of active proliferation Occasional alveoli can be seen Same magnification as fig 13

activities is afforded by the values for the exponent α, called by Huxley and Teissier (1936) the equilibrium constant, which are given in Table II. In each case the equilibrium constant given in Table II is the calculated slope of the "least squares" line, only cases where the points were well fitted by a straight line being included

TABLE II EFFECT OF ANDROGENS ON RELATIVE RATE OF TEAT GROWTH

Treatment	Equilibrium constant
Δ_5 *trans* Androstendiol*	3 65
17 Methyltestosterone (2nd phase)	3 21
Testosterone propionate (lower dilution)	2 26
Δ_5 *trans*-Dehydroandrosterone	1 77
cis Androsterone (2nd phase)	1 34
Δ_4-Androstendione	1 06
Dihydrotestosterone	1 03
Sesame oil (both groups combined)	1 08

* It is of interest to note that the teat growth promoting activity of Δ_5 *trans*-androstendiol is very much less than that of oestrone An experiment in which male guinea pigs were given daily injections of 10γ oestrone gave a value for α of 6 9

If growth of the teat and of the mammary ducts and alveoli run parallel, our results do not agree with those of Nelson and Gallagher (1936) who found that injections of androstandiol and androstendione caused marked mammary proliferation in spayed virgin rats Neither are they in accord with those of Jadassohn, Uehlinger and Margot (1938) who found that not only testosterone propionate but also androsterone, Δ_4-androstendione and androstendione caused enlargement of the teats of male guinea-pigs when applied to the teats in aqueous solutions

Certain correlations between power to promote teat growth and chemical structure were observed The fact that all the saturated androgens investigated were inactive, or nearly so, while all of the unsaturated substances except Δ_4-androstendione were active suggests that the power to promote teat growth is dependent on the presence of a double bond (Δ_4 or Δ_5) in the ring structure As a particular example, testosterone was active while dihydrotestosterone was practically inactive In addition to a double bond the presence of one hydroxyl group in either the 3 or 17 position seems necessary to confer activity, since testosterone was active while Δ_4-androstendione was almost inactive Further, comparison of the relative activities of Δ_5-*trans*-androstendiol and Δ_5-*trans*-dehydroandrosterone suggests that dihydroxy compounds are more active than hydroxyketones The activity of a 17-hydroxy unsaturated androgen is hardly affected by the introduction of a methyl group on the 17-carbon atom (compare results for testosterone and 17-methyltestosterone, fig 5)

We are grateful to Dr K Miescher and Messrs Ciba, Ltd , for the androgens used in this work and to Mr J McClemont for affording facilities for work with guinea-pigs

SUMMARY

1 In the young male guinea-pig between 150–430 g body weight the teat grows isometrically with the body as a whole

2 This teat growth ceases after castration and is, therefore, maintained by the endocrine activity of the testes

3 The unsaturated androgens, Δ_5-*trans*-androstendiol, testosterone, Δ_5-*trans*-dehydroandrosterone and 17-methyltestosterone promote teat growth, Δ_5-*trans*-androstendiol being the most potent in this respect

4 Data for the relative rates of teat growth under the stimulus of Δ_5 *trans* androstendiol, Δ_5 *trans*-dehydroandrosterone, testosterone propionate and 17-methyltestosterone give good agreement with the law of simple allometric growth

5 The saturated androgens, *cis*-androsterone, *cis*-androstandiol, dihydrotestosterone and the unsaturated diketone, Δ_4-androstendione, do not promote teat growth

6 Testosterone and its propionate were both more active in promoting teat growth when the volume of oil medium used for injection was decreased Testosterone propionate was no more active than free testosterone

7 The relative rate of teat growth caused by testosterone propionate was greater in intact guinea pigs than in recently castrated ones See 2 above

8 The activity of the androgens in promoting teat growth appears to be correlated with the presence of a double bond (Δ_4 or Δ_5) in the ring structure of the molecule, and the presence of at least one hydroxyl group in the 3 or 17 position also appears to be necessary for activity The most active substance studied was an unsaturated 3–17 diol

9 Histological examination showed that Δ_5-*trans* androstendiol, testosterone propionate and 17-methyltestosterone stimulated the mammary ducts but caused very little alveolar development

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The origin of the erythema curve and the pharmacological action of ultra-violet radiation

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(With Appendix by J S Mitchell and C B Allsopp)

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The general quantitative features of the dependence on wave-length of the production of erythema in human skin by ultra-violet radiation were established, as is well known, by the classical investigations of Hausser and Vahle (1921, 1927, cf Hausser 1928, 1934) Subsequent measurements by Luckiesh, Holladay and Taylor (1930), Uhlmann (1930), and Coblentz, Stair and Hogue (1931) have confirmed and extended the results, so that

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[*] Fellow of St John's College, Cambridge This work was commenced during the tenure of a Beit Memorial Research Fellowship

there are now available relatively complete quantitative experimental data in terms of which the pharmacological actions of ultra-violet radiation must be interpreted

The term "erythema curve", introduced by Hausser and Vahle (1927), generally refers to the curve showing the relative variation with wave-length of the "erythemal effectiveness" (which is inversely proportional to the minimal erythema dose producing a standard, just detectable, reddening), but also is used to describe the variation with wave-length of the "erythemal intensity" (degree of reddening produced by equal incident energy) (Hausser and Vahle 1927, figs 5 and 6) While the two types of curve are almost identical, the accuracy of measurement is very much greater in the former case, this alone will be considered here Typical measurements of the erythema curve (Coblentz, Stair and Hogue 1931) are shown in fig 2 (curve A_{exp}), and the measurements of various authors are summarized by Coblentz, Stair and Hogue (1931) and Ellinger (1934) The *extrapolated* long wave-length limit lies between 3150 and 3180 A, while the curve approaches the abscissa asymptotically, with very low values (Hausser and Vahle 1927, Coblentz and Stair 1933, Lomholt 1936)—less than 0 5% of the maximum—in the region 3200–3900 A The maximum of the curve lies at 2970 A and correlation of the available data suggests that the maximal error in this measurement is ± 30 A Toward shorter wave lengths the curve falls rapidly to a minimum near 2800 A, and then rises again slowly, the values at wave-lengths less than 2700 A showing great differences, according to the measurements of the different authors, however, in this region the experimental error is very great (cf Hausser and Vahle 1927, fig 6, Coblentz, Stair and Hogue 1931)

In addition to its fundamental interest, the problem of the origin of the erythema curve is of extreme importance from a therapeutic point of view, not only on account of the need for absolute dosage but also because of the relationship of erythema production to the other local changes and to the general pharmacological actions of ultra-violet radiation This latter question, together with that of the biological processes as a whole, has been considered in great detail in a number of recent reviews (Laurens 1928, Lazarus 1928, Ellinger 1934, Duggar 1936), but here the relevant quantitative data must be summarized briefly as a basis for discussion of the general significance of the erythema curve

The work of Hausser and Vahle (1921) suggested that the variation of pigmentation with wave length is very similar to that of reddening, but it is now certain that these two processes do not run exactly parallel (Uhlmann 1930), yet the wave-lengths of *maximum* efficiency for pigment production appear identical with those for erythemogenesis Further, the compilation by Vlès and de Coulon (1936) of the results obtained by previous workers on the production of malignant neoplasia by ultra-violet radiation (Roffo, 1928, 1936, Findlay 1928, 1930, Huldschinsky 1933, Wahlgren 1934),* and clinical evidence (Unna 1894, Dubreuilh 1907, Paul 1923, 1933, Matteucci 1934, Magnusson 1935, Molesworth 1933, 1937) suggest that the wave-length of maximum efficiency for carcinogenesis also lies between 2900 and 3000 A

GENERAL CONSIDERATIONS

Any mechanism suggested to explain the origin of the erythema curve must correlate the known facts of ultraviolet erythemogenesis derived from clinical observation and experiments, histological examination and photochemical investigations

From the pioneer work of Finsen (1900), the recognition of the erythemal response as an indirect capillary reaction has been discussed in detail by Krogh (1929) The experiments of Lewis and Zottermann (1926), demonstrating the "diffusion flush" after localized ultra-violet irradiation of human skin, provide direct evidence for the liberation of an active substance, the so called *H*-substance, responsible for the vasodilatation Although there is some evidence for the production of an active substance of low molecular weight which diffuses rapidly (Torok 1928), the substance responsible for the diffusion flush, "predominant in the reaction to ultraviolet light, must be one of extremely low diffusibility" (Krogh 1929)—the *H*-colloid (cf Feldberg and Schilf 1930)

Probably the most characteristic single feature of the skin reaction to ultraviolet, in contradistinction to *X* or γ radiation, is the duration of the latent period (see e g Waller 1931), which is apparently as yet unexplained

Histological examination (Keller 1924, 1928, cf Ellinger 1935, Eidinow 1935, Goldsmith 1936) shows that, apart from the vasodilatation which only appears after the latent interval, the structural changes in ultra violet erythema are usually limited almost entirely to the stratum mucosum Further, it is evident from the pigment distribution (Bloch 1927), together with the erythemal reaction in negro skin (Hausser and Vahle 1927), that the primary changes are "superficial to the site of the melanin" (cf Goldsmith 1936) In the normal skin of whites, the pigmented layer, including the basal cells and to a less extent the adjacent prickle cells,† appears to form a very efficient absorbing screen for ultra-violet radiation (Miescher 1931)

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* See Passey (1937)

† Together with the so called dendritic cells at the surface of the corium (Bloch 1927)

Hausser and Vahle (1927) regarded the physiological changes in erythema as a sequel to photochemical processes and recognized a general relationship between the erythema curve and the variation with wave-length of the absorption coefficient of human skin, the minimum of the erythema curve near 2800 A corresponded closely with the absorption maximum of skin as determined by their own measurements, which differed considerably from the earlier results of Hasselbalch (1911) Later absorption measurements by Anderson and Macht (1928), Bachem and Kunz (1929), Lucas (1931), Bachem (1931) and Angus and Taylor (1932) have confirmed these conceptions

It is well known that Ellinger (1928, 1929, 1930, 1932, 1934, 1935) has suggested that the diffusible H-substance is histamine formed photochemically from histidine, although Szendro (1931) regards it as probably iminazolyl acetaldehyde However, Bourdillon, Gaddum and Jenkins (1930) concluded that their experiments "do not support the suggestion that the erythema following irradiation of the skin is due to the formation of histamine from histidine by the simple chemical process which can be demonstrated outside the body"

A simple hypothesis, which appears consistent with these observations and is capable of quantitative formulation, is that the erythemal response is due to photochemical decomposition of some constituent of the cells of the stratum mucosum with liberation of active reaction products, which then diffuse to the region of the minute vessels of the subpapillary venous plexus of the corium and lead to vasodilatation Thus the latent interval appears to be, at least in part, a diffusion time There are two further assumptions, whose validity will be discussed below

(*a*) The stratum corneum behaves as an inert scattering and absorbing screen through which the incident radiation producing photolysis is transmitted with decreased intensity

(*b*) The pigment layer between the stratum mucosum and corium behaves as a perfect absorbing screen preventing any transmission of ultra-violet radiation into the corium *

The intensity of the erythemal reaction is assumed to be proportional to the amount of photolyte decomposed per unit area of the stratum mucosum That this is true as a first approximation is confirmed by the observations of Hausser and Vahle (1927, fig 7) that at any given wave-length for high intensities, the degree of reddening is linearly proportional, within the experimental error, to the energy incident on the surface of the skin (The duration of irradiation must be small with respect to the latent period, i e, sufficiently short that changes in the concentration of photo-decomposition products by diffusion are negligible during exposure) Thus the number of active molecules liberated is proportional to the energy absorbed in the stratum mucosum

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* The approximate validity of this assumption is shown by the work of Keller (1924, 1928), Bloch (1927), Hausser and Vahle (1927) and Miescher (1931)

In the experimental measurement of the erythema curve, large fields only are considered, i e the linear dimensions of the cross-section of the normally incident light beam are large with respect to the thickness of the stratum corneum, e g with the apparatus of Hausser and Vahle (1921) the field dimensions were 0 5–2 0 × 30 mm, while the thickness of the stratum corneum on the flexor surface of the forearm was of the order of 0 05 mm (cf Hoepke 1927, Bachem 1931, Maximow 1934) The use of large fields introduces considerable theoretical simplification

PHYSICAL THEORY*

From the point of view of light absorption the human skin can be represented by a simple "model" (fig 1) in which the epidermis is regarded as consisting of two strata of uniform thickness

(A) the stratum corneum which behaves as a scattering and absorbing screen, and

(B) the stratum mucosum, which behaves as a homogeneous solution of photolytes, in which scattering is assumed to be negligible (cf Chambers and de Renyi 1925)

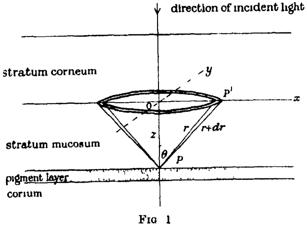

FIG 1

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* The purely mathematical steps in the argument are condensed as far as possible

The pigment layer is interposed between the stratum mucosum and the corium The stratum lucidum is probably to be regarded as part of stratum (A) together with the stratum corneum

The radiation of intensity $\mathcal{J}_0$ is incident normally on the surface of stratum A, of thickness ζ, in the positive direction of the Z axis It is required to find the energy absorption per sq cm in the stratum mucosum of thickness Z by a constituent compound of molecular extinction coefficient ϵ, present in solution in concentration C mol /l , Beer's Law being obeyed (cf Bonhoeffer and Harteck 1933)

The energy crossing normally unit area per unit time at a point P (see fig 1) in the stratum mucosum as a result of scattering from unit elementary area at the point P', at distance r from P, in the AB interface can be written in the exact form

$$I_{r\theta} = \frac{J_0}{r^2} f_\lambda(\cos\theta)\, 10^{-\epsilon C r}, \tag{1}$$

where J_0 is the intensity at P' and θ is the angle between the normal to the AB interface at P' and PP', $f_\lambda(\cos\theta)$ is the angular scattering function of stratum A (cf Rayleigh's formula, see e g Sommerfeld 1928)

Then approximating for large fields where the field dimensions $a, b \gg \zeta$

$$I_{r\theta} = \frac{\mathcal{J}_0 10^{-D}}{r^2} f_\lambda(\cos\theta)\, 10^{-\epsilon C r}, \tag{2}$$

where D is the apparent optical density of stratum A and is effectively defined by eqn (2), so that

$$2\pi\int_0^1 f_\lambda(x)\, dx = 1 \tag{2a}$$

Thus the physical measurement of D requires collection of all the light from a surface element at the AB interface with an infinitely large field

Hence it is easy to show* that the total energy crossing unit elementary area in the x, y plane at P in unit time from the whole field is given by

$$J_z = \mathcal{J}_0 10^{-D}\, 2\pi z \int_z^\infty \frac{f_\lambda(z/r)}{r^2} e^{-2\ 303\epsilon C r}\, dr \tag{3}$$

and

$$J_0 = \operatorname*{Lt}_{z\to 0} J_z = \mathcal{J}_0 10^{-D} \tag{3a}$$

Therefore the energy absorbed per unit area of the stratum mucosum per unit time is given by

$$J_0 - J_z = \mathcal{J}_0 10^{-D}\{1 - e^{-2\ 303\epsilon C z} + F(z)\},$$

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* Integrating over the AB interface divided into annuli (fig 1)

where $$F(z) = 2\pi z \int_{z}^{\infty} \frac{1}{r}\frac{\partial}{\partial r}\left[f_\lambda\left(\frac{z}{r}\right)\{e^{-2\,303\epsilon Cz} - e^{-2{\cdot}303\epsilon Cr}\}\right] dr \tag{4}$$

This expression is exact only for infinitely large fields

The evaluation of $F(z)$ requires a detailed knowledge of the scattering function $f_\lambda(z/r)$ which is not at present available However, it is possible to expand $f_\lambda(z/r)$ as a power series

$$f_\lambda\left(\frac{z}{r}\right) = K(\lambda)\sum_{n=0}^{\infty} a_n \frac{z^n}{r^n}, \tag{5}$$

and evaluate $F(z)$ using Bonnet's second mean value theorem (Whittaker and Watson 1927) Thus

$$F(z) = 2\,303\epsilon Cz K(\lambda) e^{-2\,303\epsilon Cz}\phi(z/Z), \tag{6}$$

where $Z \geqslant z$ and $\phi(z/Z)$ is an absolutely convergent series *

When $2\,303\epsilon Cz < 1$ as is the case for the stratum mucosum (see below), to a first approximation

$$J_0 - J_z = \mathscr{J}_0 10^{-D}\, 2\,303\epsilon Cz\,\{1 - 1\,15\epsilon Cz + K(\lambda)e^{-2\,3\epsilon Cz}\,\phi(z/Z)\} \tag{7}$$

On the basis of the photochemical mechanism of ultra-violet erythemogenesis, the relative erythemal efficiency W_λ at the wave length λ is given by

$$W_\lambda = \frac{(J_0 - J_z)_\lambda}{(J_0 - J_z)_{\lambda_{\max}}} \times 100, \tag{8}$$

where $\lambda_{\max}$ is the wave length of the maximum of $J_0 - J_z$ Thus to a first approximation

$$W_\lambda = \frac{(\epsilon 10^{-D})_\lambda \{1 - 1\,15\epsilon Cz + K(\lambda)\,e^{-2\,3\epsilon Cz}\,\phi(z/Z)\}_\lambda}{(\epsilon 10^{-D})_{\lambda_{\max}} \{1 - 1\,15\epsilon Cz + K(\lambda)\,e^{-2\,3\epsilon Cz}\,\phi(z/Z)\}_{\lambda_{\max}}} \times 100 \tag{9}$$

In the case when the function $\epsilon 10^{-D}$ varies rapidly with wave length, while the expression in the brackets { } varies only very slowly, $\lambda_{\max}$ can be identified with the wave length of the maximum value of $\epsilon 10^{-D}$, without invalidating the approximation, so that, in the region of $\lambda_{\max.}$, eqn (9) can be used in the simplified† form

$$W_\lambda = \frac{(\epsilon 10^{-D})_\lambda}{(\epsilon 10^{-D})_{\lambda_{\max}}} \times 100 \tag{10}$$

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* $\phi\left(\frac{z}{Z}\right) = 2\pi\left[a_0 \log\frac{Z}{z} + \sum_{n=1}^{\infty}\frac{a_n}{n\;\,n+1}\left(1 - \frac{z^n}{Z^n}\right)\right]$

† Eqn (10) is, of course, identical with the result obtained when the stratum mucosum is regarded as an elementary lamina

APPLICATION OF THE THEORY

These results are required to test the validity of the photochemical hypothesis and to identify the tissue constituents, which, if assumed to be the effective photolytes, are consistent with the observed erythema curve Thus it is essential in the case of any compound, conforming with the biological requirements

(1) to calculate the quantitative features of the erythema curve, within the experimental error,

(2) to demonstrate that the energy of radiation, which, when incident on the stratum mucosum, produces an erythemal response, can also produce, *in vitro*, photolysis with formation of decomposition products in a concentration of a reasonable order of magnitude, and

(3) if possible, to show that the active substance, whose properties are deduced from the physiological observations, can be identified with photochemical decomposition products formed *in vitro*

The probable chemical nature of the photolyte is suggested by several independent lines of evidence The *p*H in the stratum mucosum must be assumed to be approximately 7 2 (Schmidtmann 1925, Pinkus 1927, cf Chambers, Pollack and Hiller 1927, Henderson 1928, Bodansky 1934), it was observed that the extrapolated long wave length limit of the absorption spectrum of a typical protein such as ovalbumin (cf fig 2) in buffered solution at *p*H 7 2 corresponded closely with that of the erythema curve (at 3150–3180 A) There appear to be no constituents of the skin other than the *typical proteins* which are present in significant amounts and for which a similar relation holds, e g the extrapolated long wave-length absorption limit for thymus nucleic acid is approximately 3075 A (cf Caspersson, Hammarsten and Hammarsten 1934) * Further, the long wave-length threshold for photochemical decomposition of thymus nucleic acid appears to be near 2950 A (Loofborrow and Heyroth 1934)

The considerations of Krogh (1929) on the properties of the *H*-colloid provide strong experimental support—if the photochemical mechanism is correct—for the identification of the effective photolyte as a typical protein, since no other known tissue constituents have molecular weights sufficiently high for their photochemical decomposition products to include the *H*-colloid This argument can be expressed semiquantitatively The experiments of Lewis and Zottermann (1926) indicate that in the skin the *H*-colloid diffuses a distance of the order of 0 3 cm † in 26 hr (at 37° C)

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* The absorption limit for guanine nucleotide is 3070 A (Holiday 1930)

† Under approximately unidimensional conditions

By applying Einstein's diffusion equation (see e g Fowler 1929), this can be shown to correspond to a diffusion coefficient (at 10° C) of the order of 0.03×10^{-5} cm ^{2}/sec, which is consistent with that of a protein or a simple protein derivative (cf Freundlich 1926, p 547) This elementary calculation can only give the order of the diffusion coefficient of the *H* substance but is sufficient to show that in ultra-violet erythema a physiologically active macromolecule is produced

EXPERIMENTAL DATA

The required values of the molecular extinction coefficient ϵ of a typical protein were determined for a very pure specimen of ovalbumin* in solution in a standard phosphate buffer *p*H 7 2 The experimental details are being described elsewhere (Mitchell 1938, Mitchell and Allsopp 1938) and may be summarized here The absorption measurements were made, in conjunction with Dr C B Allsopp, with a standard Hilger ' Spekker" ultraviolet photometer and medium quartz spectrograph, using a condensed tungsten steel spark as light source, under the conditions in which, as described by Twyman and Allsopp (1934), the error in the measured optical density does not exceed 0 01 The iron arc lines were used as wave-length standards To avoid errors due to scattering, repeated observations were made with different lengths of absorption tube The wave length of extinction for a given equivalent density was reproducible within 5 A and at any given wave-length the maximal error in the measured values of the molecular extinction coefficient is estimated to be ± 1 5 % The values of ϵ† obtained and summarized in fig 2, curve *C*, are based on a value for the molecular weight of one Svedberg unit (34,500) as found by Svedberg and Nichols (1926), the corresponding concentration accordingly referring to protein dried in air to constant weight at 105° C, the apparent water content of the crystalline ovalbumin was found to be 13 4 % (cf Sorensen 1917, Adair and Adair 1936)

The measurement of the apparent optical density *D* of the scattering and absorbing layer of the epidermis (which corresponds anatomically to the stratum corneum, probably together with the stratum lucidum) is discussed in the Appendix The specimens of human stratum corneum used are described in the table and the measured values of D are summarized in fig 2, curves D1, 2, 3 The data for specimen 1 are taken from the work of Lucas (1931) and the corresponding values of D (curve D1) are possibly too low on account of partial clearing of the specimen by the chloroform used in its removal Specimen 2 was probably the least altered Specimen 3 was backed by a 1 mm layer of a solution of ovalbumin containing 0 091 g of dried protein in 100 c c of phosphate buffer pH 7 2 to represent the stratum lucidum, further, it is possible that scattering in the specimen may have been increased by thermal damage

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* Prepared by Dr G S Adair and kindly given by Professor E K Rideal

† Ovalbumin was selected as the typical protein on account of its general characters, further, a very pure specimen was available and solutions free from scatter could be prepared in phosphate buffer *p*H 7 2 Very few accurate measurements of the absorption coefficients of proteins appear to be available for the wave length region 2925–3050 A which is of fundamental importance in the calculation of the maximum of the erythema curve However, at these wave lengths there is exact proportionality, within the limits of experimental error, between the present measurements for ovalbumin and those of Campbell Smith (1928) for serum albumin

The form of the scattering function of the stratum corneum, $f_\lambda(\cos\theta)$, is not known quantitatively but some of its general properties can be deduced The pH of the stratum corneum is approximately 6 2 (Pinkus 1927) so that at wave lengths greater than 3100 A the observed values of D are due almost entirely to scattering The measurements of Lucas (1931) show that in the wave length range 3100–3700 A the function $(1-10^{-D})$, which measures the lateral scattering in the stratum corneum and is thus approximately proportional to $K(\lambda)$, is proportional to λ^{-1} within the limits of experimental error Within the absorption band of the keratin, which must be due almost entirely to the constituent tyrosine (Buchtala 1912), tryptophane (Marston 1928), and cystine (Morner 1901, Wilson and Lewis 1927, cf Bodansky 1934), $K(\lambda)$ probably varies even more slowly with λ since the wave-length of the maximum value of D at 2750 A is undisplaced (see also Freundlich 1926, p 383) However, in the region of wave lengths 2650–2450 A, a more rapid increase of $K(\lambda)$ with decreasing wave length is again to be anticipated

The validity of the equations (9) and (10) requires that $2\ 303\epsilon Cz < 1$ Direct measurements do not appear to be available but the value can be calculated approximately The existing data suggest that the light absorption in the stratum mucosum is probably not greater than that of a layer 0 005 cm in thickness (cf Hoepke 1927, Cowdry 1932, Maximow 1934) of a solution of a typical protein in concentration 6% (cf Kiesel 1925, Gray 1931) The measurements of the absorption spectrum of ovalbumin in buffered solution at pH 7 2 show that for 6 3% solution (calculated as dried protein) in a layer of thickness 0 005 cm, the value of $2\ 303\epsilon Cz$ is 0 139 at 2976 A, and less than 0 415 at wave-lengths greater than 2845 A and in the range 2650–2520 A, while reaching a value of approximately 0 58 at the absorption maximum at 2780 A Hence the approximation on which eqns (9) and (10) are derived appears amply justified for the human epidermis

COMPARISON OF THEORY AND EXPERIMENT

In fig 2 (curves A 1, 2, 3) is shown the variation with wave length of W_λ calculated from eqn (10) using the measurements of ϵ for ovalbumin (curve C) and those of D for the three specimens of stratum corneum (curves D 1, 2, 3), the numbers referring throughout to the specimens described in the table Comparison with the measurements of the erythema curve by Coblentz, Stair and Hogue (1931), which are included in fig 2 (curve A_{exp}), and the results of other authors (see Hausser and Vahle 1927, Coblentz, Stair and Hogue 1931, Ellinger 1934) shows a close agreement between the experimental values and those calculated The most rigorous test of the theory is the determination of the wave length of maximum erythemal efficiency The results are given in the table and the calculated mean value of λ_{max} 2975 A, is almost identical with that observed (2970 A), however, such close agreement is to be regarded as fortuitous, the probable error of the mean value being ± 15 A

The value of $\epsilon 10^{-D}$ is, of course, extremely sensitive to error in the measurement of D The maximal experimental error in ϵ is ± 1 5 %, while that in D is ± 4 % near 2970 A, the error in the measurements of D probably varying rapidly with wave length (see Appendix) Hence in the region of the maximum of the erythema curve, the error in the calculated *absolute* value of $\epsilon 10^{-D}$ is, under the most unfavourable conditions, + 28 or − 22 %, the corresponding maximal error in the individual calculations of the wave length λ_{max} from eqn (10) is ± 45 A * The magnitude of this error emphasizes the great difficulties in numerical calculation of the erythema curve and the limitations of the comparison of theory and experiment, in particular, at wave lengths greater than 2900 A, the experimental error is probably greater than errors introduced by replacing eqn (9) by (10)

The calculated curves agree with the experiments in showing a very low value in the region of 2800 A but, at shorter wave-lengths, where the measurements of different observers show great discrepancies, the curves calculated for the three specimens of stratum corneum also show great differences, further, the calculated values here are lower than any of the published data for the variation with wave-length of erythemal *effectiveness* It is interesting that curve A 1 shows a rather close resemblance to the experimental erythema curve as first determined by Hausser and Vahle (1921), and curves A2 and 3 are almost identical with the curves showing the variation with wave-length of the degree of reddening observed at intervals of 4–10 days after irradiation (Hausser and Vahle 1927, fig 6)

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* λ_{max} is assumed to be 2975 A, thus the difference between the calculated values could be attributed to experimental error in ϵ and D

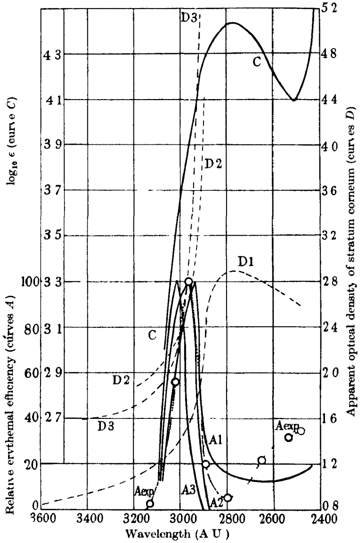

FIG 2 A_{exp} ○ ○ ○ Experimental erythema curve (Coblentz, Stair and Hogue 1931)

A 1 Erythema curve calculated for specimen 1 of stratum corneum
A 2 ,, ,, ,, 2 ,,
A 3 ,, ,, ,, 3 ,,
D 1 Apparent optical density *D* of specimen 1 of stratum corneum
D 2 ,, ,, ,, 2 ,,
D 3 ,, ,, ,, 3 ,,
C Absorption spectrum of ovalbumin in solution at *p*H 7 2

The order of the error introduced by replacing eqn (9) by (10) can be determined from the approximate values of ϵCz and the general properties of $K(\lambda)$ The values of W_λ calculated by (10) are less than 2 5 % (of W_λ) too low at 3000 A and probably less than 15 % (of W_λ) too high at 2850 A In the wave-length region 2650 − 2450 A, the term in $K(\lambda)$ is now increasing more rapidly with decreasing wave-length so that eqn (10) gives values too low, the difference between the values of W_λ calculated by (9) and (10) increasing with decreasing wave length It is probable that neglect of $K(\lambda)$ is responsible, at least in part, for the discrepancy between the observed values and those calculated by eqn (10) for wave-lengths shorter than 2650 A, however, another experimental factor may also be involved, namely the employment of different sizes of skin fields by different observers (cf Luckiesh, Holladay and Taylor 1930)

TABLE I SPECIMENS OF STRATUM CORNEUM

No	Description	Thickness mm	Calculated $\lambda_{\max}$ A
1	Removed from chloroform blister, measurements by Lucas (1931)	0 08	2940
2	Removed by vesicant plaster from flexor surface of forearm	0 048	2970
3	From scald of forearm (see text)	0 09	3014
		Mean	2975

(Probable error of mean ± 15 A)

DISCUSSION

The agreement between the calculated and experimental erythema curves is quantitative evidence consistent with the assumption of a photochemical mechanism and the identification of the photolyte as a typical protein

The minimal erythema dose (M E D) at 2970 A has been found by Hausser and Gauer (1933) to correspond to the incidence on the surface of the skin of approximately $5\ 5 \times 10^{16}$ quanta per sq cm , the correctness of this order of magnitude has been confirmed by Coblentz, Stair and Hogue (1931) At 2970 A the most reliable value of D* (specimen 2) is 2 52 so that the M E D corresponds to incidence on the surface of the stratum mucosum of $1\ 65 \times 10^{14}$ quanta per sq cm , and absorption in the stratum mucosum of not less than $2\ 45 \times 10^{13}$ quanta per sq cm Experiments† on the photo-chemistry of proteins in monolayers have shown that the absolute quantum efficiency of the total photochemical changes per "aromatic residue" in a typical protein (gliadin) spread in monolayers on $N/100$ sulphuric acid is approximately 0 5 at the wave-length 2970 A Thus the M E D leads to photo-decomposition of $0\ 33 \times 10^{-7}$ g /cm ^{2} of protein, calculated as ovalbumin* (*Biochem Handlexikon* 1933, *Tabulae Biologicae* 1926), and to a mean concentration of decomposed protein in the stratum mucosum of $0\ 7 \times 10^{-5}$ g /c c It is to be noted that this estimate of the concentration of photolysed protein is a minimum value, possibly too low by a factor of 10 (cf Lucas's values of *D*, 1931) However, on general grounds without detailed assumptions as to nature of the *H*-substance, it is suggested that this concentration of protein decomposition products is not inconsistent with the production of minimal observable vasodilatation (cf Lewis 1927, Krogh 1929, Clark 1932) Hence it appears that the direct photochemical production of *H*-substance from the proteins of the stratum mucosum is at least reasonable from a quantitative point of view

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* The available measurements suggest that differences in D alone are able to account for the individual differences in erythemal response (Coblentz, Stair and Hogue 1931)

† To be published elsewhere (Rideal and Mitchell) Cf Mitchell (1936, 1937)

The experimental fact that the principal *H*-substance is macromolecular and the suggestion, advanced in this paper, that the primary process in ultra violet erythemogenesis is photolysis of typical proteins, may be correlated in terms of some recent work showing that the photo-oxidative decomposition of typical proteins with light of wave lengths 2350–3150 A leads to the formation of proteoses and similar breakdown products, probably quantitatively under suitable conditions (Becker and Szendro 1931, Spiegel-Adolf 1934, Mitchell 1938) There appears to be conclusive evidence that histamine—like pharmacological properties—is shown by proteoses free from histamine and similar substances of low molecular weight present as impurities (See e g Abel and Geiling 1924, Geiling and Kolls 1924, Lewis 1927, Krogh 1929, p 241) Further, it is well known that certain proteoses can produce vasodilatation on intradermal injection in concentration 1 in 100,000 (Oriel and Barber 1930, Oriel 1933)

The hypothesis that the *H*-colloid is to be identified with proteoses formed photochemically offers a possible explanation of a number of the observed phenomena of the action of ultra-violet radiation, namely

(1) the local intradermal and subdermal infiltration of polymorphonuclear leucocytes (Keller 1924, 1928, Goldsmith 1936) and the general neutrophilia (Laurens 1928, Lehmann 1937),

(2) the fall in systolic blood pressure (Hasselbalch and Jacobaeus 1907, cf Frohlich and Pick 1913, Sollmann 1936),

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* The aromatic residues include tyrosine, tryptophane and phenylalanine 1 Svedberg unit (34,500) of ovalbumin contains 8 residues of tyrosine, 2 of tryptophane and probably 11 of phenylalanine

(3) the toxic reaction due to overdose and acute light stroke (Rouèche 1926, Eidinow 1935),

(4) and also though with less certainty the general reaction with which is associated non-specific immunization,

(5) the therapeutic action of ultra-violet radiation in asthma (Scott 1926, Bray 1937)

In the development of the physical theory, it has been assumed that the stratum corneum behaves as an inert screen, although it is known (Becke 1912, 1919, Sauer 1916, Bergen 1925, 1926) that polypeptide like products, extractable by hot water, can be formed by photochemical oxidation of wool keratin However, the possibility that these decomposition products can play any significant part in erythemogenesis appears to be excluded by their negligible rate of diffusion through and from the stratum corneum Water is known to diffuse through the stratum corneum very slowly (Loewy and Wechselmann 1911, Richardson 1925) but, in general, water-soluble compounds, including inorganic ions, cannot diffuse through the stratum corneum into the corium to any appreciable extent, further, it has been suggested that the stratum lucidum may act as a barrier to diffusion (Frey and Rein 1927, Whitehouse 1937, cf Whitehouse and Ramage 1933) Accordingly the diffusion of polypeptides—of molecular weight of the order of at least 1000—from the stratum corneum to the minute vessels, and lymphatics, must be extremely slow, and thus unlikely to build up a stationary concentration of active substances sufficient to produce detectable erythema The general validity of this argument is confirmed by the experimental fact that in the wave-length region 2750–2800 A, where at least 99 8% of the incident light is absorbed in the stratum corneum, with maximal photo-decomposition of the keratin, the relative erythemal efficiency reaches its minimum value which lies between 0 and 5%

The conception that ultra-violet erythemogenesis is due to photochemical decomposition of proteins introduces a number of problems of which the most important are the relationship of the erythemal reaction to pigmentation and carcinogenesis

The existence of purely photochemical melanogenesis, without the intervention of enzymes, is well known (Meirowsky 1909, Heudorfer 1921, Lignac 1923, 1929) and is consistent with the results of the photochemical investigations referred to above (cf Mitchell 1936, 1938),* there is evidence that during the oxidative photolysis of proteins, the tyrosine residues are liberated after transformation to compounds closely related to the known precursors of melanin However, there is no doubt that usually dopa oxidase plays a fundamental part (Bloch 1917, 1927) The increase of dopa oxidase after ultra-violet irradiation (Bloch 1927) must be regarded as a "biological" response to the photochemical changes, and the involvement of a series of secondary biological changes is consistent with the observed qualitative (but not quantitative) parallelism between the variation with wave-length of erythemogenesis and of pigmentation

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* See also Mitchell, Rideal and Schulman (1937)

The apparent resemblance between the effectiveness of the different wave-length regions for the production of erythema and of neoplasia is of very great interest Carcinogenesis by ultra-violet irradiation is usually preceded by repeated severe erythemal reactions in normal human skin (Dubreuilh 1907, Beck 1933, Paul 1933), in laboratory animals (Putschar and Holtz 1931, Roffo 1936) and in the abnormal skin of xeroderma pigmentosa (Dalous and Constantin 1904, Haxthausen 1919, McCarthy 1931), so that the relationship to erythemogenesis may signify that the neoplasia is related to chronic inflammatory and/or atrophic changes The possibility that carcinogenic agents may be liberated from proteins—and also from sterols (cf Kawaguchi 1931, Roffo 1936)—is being investigated experimentally in conjunction with Dr D L Woodhouse of the General Hospital, Birmingham (see *Annual Report of the British Empire Cancer Campaign*, 1937, p 84) *

Finally, I wish to express my sincere thanks to Professor E K Rideal, F R S, for his help and advice

APPENDIX

By J S Mitchell and C B Allsopp

The measurement of the (optical) density of the human stratum corneum

For calculation of the erythemal efficiency, the apparent optical density D of the stratum corneum must be measured under conditions in accordance with the definition given in eqns (2) and (3 a), the physical meaning of this is that, with an infinitely large field of normally incident light of intensity $\mathscr{J}_0$, the total energy of the forward scattered light† crossing unit elementary area in unit time is given by $\mathscr{J}_0 10^{-D}$ The experimental fulfilment of the conditions is qualified by the restriction that only that part of the light falling on the slit of the recording spectrograph, which is contained in a cone whose vertical angle is equal to the angular aperture of the collimating lens, will ultimately be measured However, the intensity of the components scattered at large angles must be assumed to be small

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* As yet, with negative results

† Including that "transmitted" with loss of intensity by absorption

A method of measurement with scattered incident light has been described by Lucas (1931) and the apparent values of D obtained were used, in addition to our own, for calculation of the erythema curve (fig 2, curves $A1$, $D1$) This method cannot be regarded as identical with the "large field and normal incidence" method used here, and may give lower values of D at all wave-lengths, however, if as suggested above, the intensity of large angle scattering varies only very slowly with wave length in the region of the absorption band of keratin, the systematic error introduced into the calculation of the wave-length $\lambda_{\max}$ is almost certainly less than the experimental error

In the present measurements, the radiation was normally incident on the specimen of stratum corneum over a circular field of diameter 1 cm and the scattered light collected to the full aperture of the spectrograph ($f/10$)

The specimen, pressed between quartz plates of the highest optical transparency, was placed immediately in front of the slit of the Hilger Medium Quartz Spectrograph and was illuminated by light from a condensed tungsten-steel spark at approximately six centimetres distance Under these conditions all the light recorded on the plate either had been transmitted directly along the axis of the instrument or had been scattered into the slit from neighbouring portions of the specimen, and the only losses involved the weak components scattered at large angles The systematic error due to variation of the angular scattering function with wave length is assumed to be small The apparent densities were determined by Henri's method as described by Allsopp (1934) The Ilford Auto-Filter plates used had a "time factor" of 1 1 The experimental error in the measurements of D (see fig 2, curves $D2$, $D3$) in the region of the *maximum* of the erythema curve is certainly less than 5 % and probably does not exceed 2 5 % For wave-lengths shorter than 2900 A the error may rise to the order of 10 % because of the very long exposures involved in measuring the high densities in this region, so that there may be a more serious error in the calculated values near the *minimum* of the curve at about 2800 A, this is clearly of negligible importance in determining the position of the *maximum* (see Table 1) We hope to investigate the value of the scattering function and its relation to D later

SUMMARY

The measurements of the erythema curve and the relevant known facts of the reaction of the human skin to ultra-violet light are reviewed The

possibility of a photochemical mechanism is discussed and developed theoretically

Quantitative evidence is presented to show that the erythemal response is due to photochemical decomposition of typical proteins in the stratum mucosum by the light transmitted through the stratum corneum, this latter behaves as an inert scattering and absorbing screen The photochemical changes are limited to the stratum mucosum by the distribution of pigment The latent period is, at least in part, the time required for the diffusion of active photo-decomposition products to the minute vessels

The form of the erythema curve is calculated, within the limits of experimental error, from absorption measurements of a typical protein and of the human stratum corneum The calculated mean value of the wavelength of maximum erythemal efficiency is 2975 (± 15) A, in close agreement with that observed The known minimal erythema dose is consistent with the formation of photo-decomposition products *in vitro* in concentration of a reasonable order of magnitude

It is suggested that the *H*-colloid may be a proteose formed by oxidative photolysis of proteins

The relationship of erythemogenesis to pigmentation and carcinogenesis by ultra-violet radiation is briefly discussed

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Address of the President Sir William Bragg, O M , at the Anniversary Meeting, 30 November 1938

According to our honoured custom I preface this annual address by references to those of our Fellows whom death has taken from us during the past year

ARTHUR HUTCHINSON (1866-1937), Emeritus Professor of Mineralogy in the University of Cambridge and lately Master of Pembroke College, was elected a Fellow of the Royal Society in 1922 He had served on the Council from 1932 to 1934 and was a Vice-President for the year 1933–4

While still young Hutchinson made a name for himself by brilliant researches on the chemistry and crystallography of stokesite and other minerals He devised a stereographic projector and a slide rule which are much used by students of crystallography and he was skilful in the construction of crystal models and lecture-room apparatus As a teacher in the University of Cambridge he was highly successful though it was not until 1926 that he became Professor of Mineralogy, his work had long been of professorial standing When the use of X-ray methods from 1914 onwards opened up a new crystal science, he at once attached himself to its ways and aided its development He designed his instruments afresh He devoted his energies towards the organization of his Department to meet the altered needs in teaching and research He was a devoted servant to Science, an excellent investigator, an able teacher and one of the kindest and most loyal of friends

GEORGE HENRY FALKINER NUTTALL was born in San Francisco in 1862 He was educated in England, France, Germany and Switzerland, and to this cosmopolitan upbringing he owed his ability to speak several languages He returned to America in 1878 and obtained the degree of M D at the University of California in 1884 Nuttall's scientific work covered a very wide field, in addition to several books, he published numerous papers on bacteriology, serology, hygiene, tropical medicine and parasitology His outstanding achievement was the discovery that the blood and other body fluids of animals possess bactericidal power, a discovery which laid the foundations of all work on serum antitoxins In 1904 he published his classical monograph *Blood immunity and blood relationship*, this investigation has clearly demonstrated the existence of a distinct similarity in chemical structure of the blood in animals which are related phylogenetically

Nuttall was elected the first Quick Professor of Biology in Cambridge and he held this Chair until 1931 He was elected a Fellow of the Royal Society in 1904, he was Sc D of Cambridge and had received honorary degrees from five other universities

In 1901 Nuttall founded the *Journal of Hygiene* and in 1908 *Parasitology*, both of which he edited with great distinction He died suddenly in London on 16 December 1937

LOUIS NAPOLEON GEORGE FILON died on 29 December 1937 at the age of 62 Apart from three years at King's College, Cambridge, as one of the early "advanced students", his whole academic life was spent at University College, London His contributions to applied mathematics were in the classical field and particularly in the theory of elasticity His patience and thoroughness and his skill in algebraic manipulation qualified him well for work in this field, and his physical insight led him to choose problems of importance and significance His most notable contributions were the principle of "generalized plane stress" which he enunciated early in his career, and the subsequent application of this principle in the development of photo-elasticity as a practical means of expressing stress distribution His researches are contained in nearly sixty books and papers published throughout the course of a very busy life, for he was also an able and enthusiastic teacher and took a very active part in the administration of his University

He was elected a Fellow in 1910 and served as Vice-President of the Society for 1936 and 1937

ALFRED BARTON RENDLE, who died in January of illness contracted on his way to attend the Jubilee Session of the Indian Science Congress, was a botanist esteemed abroad and at home for his knowledge, judgment and helpfulness to others

When Rendle entered St John's College, Cambridge, in 1883, an interest in plants, encouraged at school, led him to study botany He graduated in 1887 and was appointed in 1888 an Assistant in the Botanical Department of the British Museum In a very short time the quality of his work had brought him a request from Berlin to write a monograph on a family of plants for the *Pflanzenreich*, and similar appeals on behalf of the *Index Florae Sinensis* and the *Flora of Tropical Africa* in this country Permitted by the Trustees of the Museum to comply with these requests, Rendle showed his readiness to help others and incidentally added to the prestige of the department, to the Keepership of which he was promoted in 1906 His judgment was subjected to a more searching test, and earned correspondingly enhanced appreciation, during his service from 1905 till 1935 as an active member of the Editorial Committee of the International Laws of Botanical Nomenclature The Royal Society, to which Rendle was elected in 1909, has reason to know that systematic botany was not the only subject to benefit from his exact knowledge and impartial judgment for, from 1913 till he died, his service on its various committees was almost continuous and he was a member of the Council during 1929–31

Sir James Crichton-Browne, who died on 31 January 1938 at the age of 97, was the oldest Fellow of the Royal Society For many years he occupied a unique position in the profession of medicine He followed the speciality of his father, who was a distinguished psychiatrist, and devoted the greater part of his life to the study and the welfare of the insane At a relatively early age he was appointed to the charge of the West Riding Asylum at Wakefield and at once instituted a study of the brain and its disorders The pathological laboratory which he established there was the first of its kind in England, and under his guidance Wakefield became one of the most important centres of neurology in this country The Reports which he published "to give stimulus to the arrangement and elaboration of material hitherto buried in case books" contained important papers by Ferrier, Hughlings Jackson, Clifford Allbutt and others, who at that time had no more suitable medium of publication Later he took a major part in the foundation of *Brain* After he had been appointed, at the age of 35, Lord Chancellor's Visitor in Lunacy, his work was mainly administrative, but various reports, as that on the influence of early education on mental health, showed that he retained his earlier interests He took great interest in the work of the Royal Institution of which he was Treasurer for thirty-seven years He was elected to the Fellowship of the Society in 1883

George Ellery Hale (1868–1938) Hale's memory is to be cherished as that of a most noteworthy leader in the recent advances of our knowledge of the phenomena presented by the surface of the sun By his invention of the spectroheliograph in the early years of his youthful studies, he opened up a new method of recording photographically the contribution made by any selected monochromatic solar radiation (and hence of any chosen chemical element) to the pictures of the features of the sun's surface as scrutinized in ordinary telescopic vision

In 1895, he undertook the initiation and direction of the Yerkes Observatory of the University of Chicago, and carried the work forward for ten years with marked success Before he had reached the age of 27 he had gained recognition as a leader, without a trace of self-assertion Failure of health and the desire for better physical conditions of astronomical observations led him to California, instigated by the prospect of the foundation, by the Carnegie Institute of Washington, of a mountain observatory for intensive study of the sun On the summit of Mount Wilson he established a solar observatory with novel equipment of powerful instruments With these he discovered definite indications of vortical motions in and about sunspots, and was led thereby to look for signs of the Zeeman effect in the broadened lines of the sunspot spectra The success of his design of the 60 ft tower telescope with a 30 ft spectrograph sunk in a vertical shaft beneath the tower, led him to design the 150 ft tower telescope with a spectrograph of 75 ft in a shaft 80 ft deep With this equipment specially designed for the purpose he proceeded to detect and measure the intensity of the general magnetic field of the sun, analogous to the earth's magnetic field

The remarkable success attained by the use of the 100 in reflector presented by J D Hooker, in studies of the remoter nebulae encouraged Hale to take a prominent part in the colossal enterprise of the 200 in reflector, which is now under construction It is to our deep regret that it has not been given to Hale to see its completion Hale became one of the Foreign Members of this Society in 1909, and was awarded our Copley Medal in 1932

EDWARD MEYRICK, who died on 30 March 1938, was a very remarkable man and exerted an influence both as a classical master at Marlborough College and as a naturalist—especially as an entomologist—far wider than he himself probably realized During his active life of over 83 years, he became recognized as the world's authority on the microlepidoptera He continued working until within a few days of his death and retained to the end his firmness and accuracy of hand and eye

It was no small satisfaction to him to see some of the boys, whose scientific interests he had fostered at Marlborough, become eminent scientists of later days

He was educated at Marlborough and at Trinity College, Cambridge, where he was a Classical Scholar After nearly 10 years teaching work in Australia and New Zealand, where he also studied the microlepidoptera in his spare time, he returned to Marlborough as a classical master in 1887, and was the inspiring President of the Natural History Society for 25 years

An indefatigable collector and an accurate observer from his schooldays, he found the microlepidoptera an almost unexplored field for study, and during 60 years of intensive work described some 20,000 species, besides hundreds of new genera and several new families His magnificent collection of over 100,000 specimens, minutely labelled, together with his own excellent water-colour drawings, and a complete index in his small, clear handwriting has been presented, under his will, to the Natural History Museum

Meyrick revolutionized the classification of the lepidoptera, basing his rearrangement on structural characters, and enunciated three principles, now known as "Meyrick's Laws", by which he was convinced the systematist must be guided

He became a Fellow in 1904

JAMES ERNEST MARSH, who died on 13 April 1938, aged 77 years, was associated all his life with Oxford From 1886 onward he was an active worker in organic chemistry, notably in the field of camphor and the related terpenes Early to grasp the principles of space isomerism, he found a virgin field in these complex ring structures, and, in the case of the camphoric acids, was the first to obtain optically active, *dextro*- and *laevo*-varieties for both the *cis* and *trans* forms of geometric isomers

In association with his students, notably with J A Gardner, he made a very original and still interesting, though unsuccessful, attack on the problem of the constitution of camphor and turpentine

Stone preservation was the subject of a highly original little memoir by Marsh in 1926 Regarding the decay as due most frequently to bacterial life in the stone, his remedy was based on sterilization by weak alkalies An occasional good wash with soap and water he regarded as beneficial for the outsides of buildings as of men

In his *Origins and growth of chemical science* (1929) he stressed the claims of the British workers, Black and Boyle, on salt formation and the fixation of gases, as the real founders of a science of chemistry

Marsh was a keen yachtsman and good company both in work and leisure His original and independent outlook will be remembered affectionately by the many who came under his influence at Oxford

The Cavendish family has always been conspicuous for the importance of its contribution and great devotion to public affairs VICTOR CHRISTIAN

WILLIAM CAVENDISH (1868–1938), ninth Duke of Devonshire, was actuated fully by this family spirit

Part of his early manhood, after Eton and Trinity, was spent in London offices and chambers in pursuit of that particular type of knowledge which he knew would fit him more fully for the work of administering the vast estates which it was his destiny to possess

His political life began when, as Mr Victor Cavendish, he was returned in 1891 as member for West Derbyshire in succession to his father—a seat which he held until he succeeded to the Dukedom of Devonshire in 1908

The Duke's interest in the north of England found one of its outlets in his Chancellorship of the University of Leeds There he continued a family association with the original Yorkshire College of Science, founded in 1874 The first President of the college was his uncle, Lord Frederick Cavendish, in whose honour one of the original chairs of the university bears the name of Cavendish He had been High Steward of Cambridge University from 1923

One of the Duke's empire services was his very successful Governor-Generalship of Canada from 1916 to 1921

After Canada post-war England claimed his unremitting attention A year in Mr Bonar Law's government as Colonial Secretary and a close connexion with the production of the Empire Exhibition at Wembley must have taken up a very great deal of his time, and it is characteristic of his methods that he quietly took on his shoulders a very large part of the financial strain of the Wembley Exhibition as principal financial guarantor without the public of the time being aware of it

It was in 1925 that the strain of public life took such toll of his energy that a sudden collapse compelled him to limit his public work very strictly He was elected to the Fellowship of the Society in 1914

ARTHUR EDWIN BOYCOTT, who died at the comparatively early age of 61, was an eminent pathologist and also a distinguished naturalist He received his early scientific education at Oxford and completed his medical studies at St Thomas's Hospital Subsequently he held pathological posts at the Lister Institute and at Guy's Hospital He was Professor of Pathology at the University of Manchester 1912–14, and at University College Hospital, London, from 1914 until 1935, when he resigned owing to ill health

He was a partner with Haldane in researches into the causation of miners' anaemia, of caisson disease, and of the accidents attendant on deep diving and into the pathology of mountain sickness The practical applications of these researches have been of great advantage to industry, increased materially the depth at which divers can operate with safety and have led to a better understanding of acclimatization to high altitudes

Boycott's best known researches in pathology are those upon the volume of the blood and regeneration of blood corpuscles in anaemias produced by various causes

Boycott was an ardent and single-minded investigator, whose influence upon his contemporaries was good and great He was an artist in fine expression and gave a number of stimulating addresses As editor of the *Journal of Pathology* he rendered valuable service to British pathology

His work in the domain of natural history was equally esteemed by zoologists He was a recognized authority on the ecology of the British land and fresh-water molluscs, and his genetical researches into the inheritance of sinistrality in the pond snail *Limnaea peregra* published in the *Transactions* of our Society in 1928 is regarded as one of the classics of genetical research

Boycott was elected a Fellow in 1914 and served on the Council 1929–31

JOSEPH WILLIAM MELLOR, born at Huddersfield in 1869, spent most of his early days in New Zealand After graduation at the University of Otago, he was awarded an 1851 Exhibition Scholarship, which took him to the University of Manchester in 1899 Under Dixon he completed a number of researches, perhaps the most important being concerned with the combination of hydrogen and chlorine His long association with the ceramic industries began with his appointment as lecturer in pottery manufacture at Stoke-on-Trent He was the first Principal of the North Staffordshire Technical College, built in 1914

Mellor played a conspicuous part in the progress of clay technology in Great Britain He raised the status of the Ceramic Society from that of a local association to that of an international institution He was quick to realise the importance of refractory materials to the well-being of an industrial nation In 1909 he was closely associated with the formation of the Refractories Committee of the Institution of Gas Engineers which has been responsible in Great Britain for pioneer work into the properties of refractory materials

In 1937 he completed a monumental work, *A Comprehensive Treatise on Theoretical and Inorganic Chemistry*, in sixteen volumes The achievement is almost beyond comprehension He wrote every word and every reference

Mellor was a man of great courage, ability and pertinacity He was greatly liked perhaps, to those who knew him, his greatest attribute was his loyalty to his work and to his friends He became a Fellow of the Society in 1927 He died on 24 May of this year

The official notification of the election of JOHN JACOB ABEL to the Foreign Membership of the Royal Society was delivered at his house in Baltimore on the day of his death, on 26 May, a week after he had attained the age of 81 years He was born near Cleveland, Ohio, into a family which came originally from the German Rhineland He graduated at the University of Michigan and subsequently continued his studies in a number of the leading medical centres of the continent of Europe He returned to the United States to hold, in succession, the Chairs of Pharmacology at Ann Arbor and Baltimore, the latter for 39 years Of the researches in which he was engaged throughout his long career, those on the chemistry of the hormones, from epinephrine to insulin, will probably leave the most permanent mark in the history of science He played a leading part in establishing both biochemistry and pharmacology as separate subjects of teaching and research in the United States of America, and had a powerful influence in promoting the development there of all branches of medical knowledge by experimental research He was busily engaged in new researches, undertaken in his retirement, right up to the onset of his last brief illness

WILLIAM ARTHUR BONE (1871–1938), a man of great energy and forceful character, made many important contributions to the chemistry of combustion, the constitution of coal and the analysis of reactions in the blast furnace His work was characterized by great thoroughness, pertinacity, and experimental care, and was rewarded by the discovery of many interesting new features in the behaviour of gaseous mixtures on combustion His early work on the slow combustion of hydrocarbons (1902–6) led him to hold tenaciously the theory that their oxidation occurred through successive stages of hydroxylation, to which view his experiments on the high temperature combustion of the simpler hydrocarbons lent further support As Livesey Professor at Leeds University, he developed the technical applications of surface combustion in collaboration with the late C D McCourt and was awarded the Howard Potts Gold Medal of the Franklin Institute in recognition of the advance in technology so made

As Professor of Chemical Technology at the Imperial College of Science, he built up a school of research, and with the assistance of his collaborators studied combustion at high pressures, reaching an explosion pressure as high as 10,000 atm He discovered phenomena relating to the effect of nitrogen in carbon monoxide explosions and proved that carbon monoxide could burn even when completely dry He studied the initiation of detonation, the helical propagation of the head of detonation, and the effect of electrical fields on the propagation of flame He started a series of researches on the constitution of coal in which field he became the foremost investigator He proved the benzenoid character of the coal substance He returned in later years to his studies on the slow combustion of hydrocarbons, publishing a series of valuable papers He was elected to the Society in 1905, gave the Bakerian Lecture in 1932, and in 1936 received the Davy Medal

WILLIAM WALLACE CAMPBELL'S outstanding contribution to astronomy is his determination of the radial velocities of the stars In 1896, when the radial velocities of only a few of the brightest stars were known, he designed a suitable spectrograph for the great telescope of the Lick Observatory, with which results of surprising accuracy were obtained He embarked on the ambitious programme of determining the radial velocities of 2770 stars of visual magnitude greater than 5^m 50, and carried this through It involved an additional observatory in Chile under Dr Wright's direction with a similar spectrograph on a 36-in reflector

The observations disclosed a large number of spectroscopic binaries The peculiarities of the orbits of these stars threw light on the evolution of double stars In addition they showed that the stars of earliest spectral type had large masses

The radial velocities were naturally discussed to give the motion of the sun among the stars The direction agreed substantially with that found from proper motions, but the velocity of 19 km per sec was determined with much greater precision Incidentally Campbell made the discovery that the early type or hottest stars had an apparent recessional velocity of 4 km per sec

Campbell made six expeditions to observe solar eclipses and devised a beautiful method of showing the reversal of the Fraunhofer lines In 1922 with Trumpler he confirmed the verification of Einstein's prediction made by the British observers in 1919

He was elected a Fellow in 1918, he died in 1938 at the age of 56

ALFRED EDWIN HOWARD TUTTON, who died on 14 July 1938, was elected a Fellow of the Society in 1899 at the age of 34 His scientific life was mainly devoted to a single research begun when he was a young lecturer in chemistry under Sir Edward Thorpe, and continued for 40 years For 25 years his scientific work was carried on concurrently with the duties of H M Inspector of Technical Schools in London, Oxford and Devonshire successively In each of these centres he established a private laboratory in which he determined the changes in form and physical properties which are produced in the crystals of an allied series of compounds by the replacement of one metal by another For this purpose he investigated a vast number of crystals of sulphates and selenates and their double salts It was a striking example of persistence, patience and successful achievement

After his retirement from the inspectorship in 1924 he returned to another task begun many years previously and involving the greatest patience and accuracy, namely, the evaluation of the length of the imperial standard in terms of wave-lengths of light

Crystallography was the passion of his life He published a valuable and comprehensive treatise on the subject He also published some lighter and more popular work on ice and snow, to the study of which he devoted much time during holidays spent in the Swiss Alps

All Tutton's work was characterized by great experimental skill and fine finish

In the death of ERNEST WILLIAM BROWN (1866–1938) we have lost one who embodied the spirit of the older mathematical astronomy, that concerned with the motions of the planets His name will stand as the culmination of a list, beginning with Newton and Laplace, of those who have attempted to explain the detailed motion of the moon It is a problem that he mastered completely, though his work involved an almost unbelievable degree of elaboration including the evaluation of some ten thousand separate terms and the rejection of a still greater number His Lunar Tables are now universally accepted as complete for the time being, and the fact that there are still minute discrepancies is left to provide future workers with interesting fields for speculation In his later years he attacked with much success problems connected with the very peculiar motions of some of the minor planets Brown was by birth a Yorkshireman He learnt his subject at Cambridge, but soon afterwards removed to America It was at Haverford that he began his great work on the moon and it was concluded when he later removed to Yale, where the remainder of his life was spent He had the very rare distinction of being a full member both of our Society and of the National Academy of Sciences

BASIL MOTT, born on 16 September 1859, died on 7 September of this year When a young man Mott was an assistant to Greathead during the construction of the City and Southwark Subway, which formed the first part of the City and South London Railway This work involved the pioneering use of the Greathead shield So much were his skill and energy relied on that he became Greathead's partner, and he operated the railway for the first eighteen months after it was opened in December 1890 He afterwards joined Sir Benjamin Baker in the construction of the Central London Railway, which was opened in 1900 These works and others which followed were the means of establishing the practice of deep tube tunnelling as an engineering method, and the process was thereafter applied in many other cities throughout the world

Mott was responsible, at first in partnership with Sir Benjamin Baker, and later in sole charge, for the widening of the Blackfriars Bridge completed in 1909 In later years with his partners in the firm of Mott Hay and Anderson he was responsible for a number of important bridges Possibly the greatest of the works with which he was associated, and one which fully absorbed his time and his energies in his later life, was the Mersey Tunnel, the largest under-water tunnel in the world, completed in 1934 In 1925 he was chairman of a committee of engineers and architects which took the necessary steps for the preservation and safety of St Paul's Cathedral

He was elected to the Royal Society in 1927

The Society records with regret the death of H R H PRINCE ARTHUR OF CONNAUGHT, K G, which took place on 12 September 1938 Prince Arthur was elected a Fellow on 26 March 1914 under the Statute providing for the election of Princes of the Blood Royal he attended a meeting of the Society and signed the Charter Book on 25 June of the same year

ROBERT LUDWIG MOND (1867–1938) was the second son of Dr Ludwig Mond, F R S Like his father he was devoted to scientific research and particularly to the advancement of industrial chemistry When young he assisted in the investigations leading to improvements in the production of zinc by the electrolysis of zinc chloride and to the discovery of nickel carbonyl This and later work on other carbonyls were notable achievements Yet his greatest contributions to the advancement of science were due to the enthusiasm, good judgment and generosity with which he promoted other work than his own Under his father's early direction he designed and equipped the Davy Faraday Laboratory of the Royal Institution, and afterwards gave liberally to its support He took a great interest in the foundation of the Maison de la Chimie in Paris, contributed largely to its funds, and served as a member of its Council He made generous gifts to the Norman Lockyer Observatory at Sidmouth He carried on research into various problems of physics, chemistry and agriculture in his laboratory at Combe Bank

Robert Mond did much for the archaeology of Egypt and Palestine His discoveries during 30 years' digging in Egypt now enrich many museums, the treasures to be found in his own collection at his house in Cavendish Square make it one of the finest in private ownership

The value of his scientific and archaeological work was acknowledged by the distinctions which he received from learned bodies in many different parts of the world He was elected to our Fellowship a few months ago

The past year has included many events which might well be mentioned in this annual address One of them has, however, elbowed out most of the rest The two numbers of *Notes and Records* which have begun a new enterprise contain interesting and informative accounts of our doings since last November I believe that Fellows have appreciated this social and intimate addition to the publications of the Society, and that they will join with me in an expression of our gratitude to the officers who have produced it, and especially to the one who has on this, as on so many occasions, taken the lion's share of the work The doings of the Society are noted and recorded in this periodical more effectively than I could do in a Presidential address

I must now speak of an event which is surely uppermost in the minds of all who have received the nominations of Council for next year We are to lose the services of Sir Frank Smith You may be surprised at his decision not to seek renomination when we would gladly have kept him for one more year It happens, however, that two more of our officers must step down at the end of next year, and Sir Frank has pointed out that if three were to leave at one time the business of the Society might be seriously affected We cannot, therefore, ask him to stay We must take advantage of the opportunity thus given us to tell him how grateful we are for the work he has done for the Society His secretaryship has been distinguished by a rare exhibition of capable service The place which the Society fills in public life, and its harmonious co-operation with other institutions are largely due to his wisdom and tact and his power to unite workers in a common effort Indeed, it is impossible to think of what he has done for us, without reflecting at the same time that he has exercised his talents in a wider field, and that in the words that may seem simple but are full of meaning, he has been and is still I am glad to say a great public servant

I should like to add a reference to one very interesting matter As the result of a discussion between Dr Kruss, head of the Prussian State Library in Berlin, and myself, when we attended the International Documentation

Conference at Oxford in September last—a discussion initiated by Dr Kruss—I have lately received a letter from Dr Bosch, President of the Kaiser Wilhelm Gesellschaft, inviting the co-operation of the Royal Society in some scientific enterprise which would advance science and, at the same time, promote understanding and good will Dr Bosch asks that a few representatives from this side should be the guests of the Kaiser Wilhelm Gesellschaft for a week during this winter and hopes that we would play the part of hosts in return During these interchanged visits, lectures would be given and consultations would be held, and it is reasonable to hope that some plan of a joint work would emerge I feel sure that this gesture of friendliness will receive a warm welcome from the Fellows of the Society

I will now say a few words on a subject which seems to me to exhibit a greater importance the more closely it is examined

In recent years the very great increase in the output of the results of research has placed our scientific societies in a difficult position There is correspondingly more to be published, and, at the same time, publication costs have increased Consequently, the societies' finances are overstrained, and publication of worthy material is restricted The Royal Society receives a Government Grant for publication purposes, far the greatest part of which is passed on to other Societies The grant is sufficient to meet only a portion of the applications for assistance that are made

There is another reason why the increase in output presses hardly on our societies In order that a proper standard of merit shall be maintained, it is customary to submit each paper, when it is sent in to one or more referees Now the number of possible referees is limited, and is even becoming relatively smaller as specialization increases I may take this opportunity of expressing the gratitude of the Society to those Fellows who spend so much care on the examination of the papers submitted to them The Officers realize regretfully that they ask them for a considerable fraction of their time and energy

Our shelves display the effects of increase As we look at the long rows of bound periodicals in our studies, we realize not only that many years have slipped past since we began to collect them, but also that their very appearance is an item of history A long time ago they were comparatively small and thin But they grew they shrank during the bad years of the war, but soon resumed their increase, and of late they have swollen till they have become unmanageable and must needs be divided Still the papers come pouring in, and the rate of flow even increases It is all to the good, and a healthy growth which we do not want to check, but there are practical difficulties which invite an attempt to solve them

It seems to me that when we try to consider this matter from points of view all round it, we are driven to seek for a precise answer to the questions, "Why *do* we publish? Why do we submit papers and why does the Society print them, if they are good enough?"

When a man submits a paper to the Society he is, in the first place and quite rightly, anxious for the satisfaction of showing what he has done to those who will understand it Another reason which has certainly grown in strength of recent years is that he wants to establish his reputation and position Doubtless, he has also the wish that his work may be of service, though this desire may be relatively less obvious even to himself To the Society the opportunity for service is the principal reason, for it cannot be held that the Society exists in these days, only or even mainly, for the pleasure and profit of its Fellows

The existence of our Society depends on our belief that knowledge is to be obtained by experiment In that belief our founders of nearly three hundred years ago organized the rules and procedure on which we have acted ever since Their foresight has been amply justified, but they could not have imagined how far experiment would carry their successors and others who have held the same belief We have been led to the discovery of a natural world of vast extent, and without limits that we can see Every part of it is of interest and every item of knowledge that we find there has a bearing on what we think and do As we see and know, the results of the exploration have already been immense, modern life has been profoundly affected thereby The natural knowledge which the Royal Society set out to improve has become one of the greatest influences of these times The Society and other bodies and individuals that have followed in its footsteps have, therefore, become possessed of certain most important opportunities which are indeed duties One of these is still, as it has always been, the encouragement of research, wisely conceived and well directed A second is the preparation and presentment of the results of research, so that they can be sufficiently appreciated, and can be incorporated with understanding into every activity, intellectual or physical, to which they apply We are not, as discoverers, responsible for the uses that are made of our discoveries We ought not to claim, in my opinion, to be given the direction of affairs in which our discoveries play a great part simply on account of that fact But we are, at least, bound to see that our acquired knowledge is rightly stated so that it can be rightly used We may also, as scientists, feel that we ought to help in putting knowledge to good use This duty is actually discharged by numbers of men in these times

It is curious to think that the feeling of responsibility is of recent growth

There was even a time when a discovery could be considered a private possession, to be withheld from general knowledge if thought fit Newton, Hooke, Cavendish and many others were often in no hurry to give to the world what they had found, they were influenced by indifference, by fear sometimes, even by impish playfulness, as when truths were hidden in anagrams They did not then look upon natural knowledge as an inheritance belonging to mankind, which, when discovered, ought to be shared

Rumford, at the end of the eighteenth century, was one of the first to accept and proclaim his responsibility, and act upon it, when he tried to apply the laws of heat to the economy of fuel His first attempt in London was made at the Foundling Hospital The Royal Institution, in its first form, was his attempt to give concrete expression to his idea The founders of the British Association were urged in their work by a similar motive and were to some extent under the belief that they were repairing a defect of the Royal Society The vigorous action of the Association in these days is a most commendable extension of its original purpose The Royal Society undertakes a special part of the general responsibility It is concerned with affording opportunities for the publication and discussion of original discovery, it also encourages discovery by the administration of funds entrusted to it for that purpose And, of course, it represents officially the scientific activities of the nation, acting as an adviser on such matters in public affairs In this field it has full opportunity for the exercise of all its powers This limitation of service does not free the Society from the need to attend always to the one great purpose, more fully realized of recent years, that of opening to mankind the new fields of natural knowledge The new fields are not merely to be penetrated, they are also to be made public There is, of course, a well-known saying that praises knowledge because it is useless This cryptic statement would seem to be an ethical injunction to the researcher, it certainly does not mean that the most desirable knowledge is of no use to any one but the discoverer

Even within the range of activities to which the Royal Society confines itself, in which contact with the general public is not often made, regard must be paid to the future exposition of discovery Because the results of research have become so voluminous and so important, the manner of stating them, of arranging and storing them has also become important We have to bear this in mind when we think of the arrangement on our shelves

At the present time, the volumes of our *Proceedings* and *Transactions* have a certain resemblance to a building site on which the contributors of materials have shot their goods and it must be said that the shooting is often done without regard to convenience of subsequent handling He who would construct for himself a whole building finds it difficult to make use of the materials provided. Anyone who has completed a piece of research and hopes that it may be accepted and published by the Royal Society or any Society of learning, is in the first place at pains to describe what he has done, the methods he has used and the inferences that he draws from his results. The manner of telling his tale will depend on the purpose he has in mind. He may think that he ought to set out his facts in full detail, so that any reader who is interested shall at least find all that he wants. If he is on the threshold of research work, he will often be influenced by the desire to prove his knowledge and ability, and may be led to make his tale long and complicated. It is very likely that he has not before him a mental picture of the man for whom he is writing; if he has, it is probably that of an expert, who is working in the same narrow region as himself, who knows all the technical terms and is familiar with the hidden difficulties of the subject (which, therefore, must be discussed in full), and will pick holes if he can. It is true that writing of this kind is of the highest value: expert must talk to expert.

Nevertheless, such writings make dull and difficult reading for the great majority of those who are interested in scientific discovery. How few there are who can critically study a paper of a certain type is only too well known to our secretaries and the chairmen of our Sectional Committees, who must find referees to judge its merits, and to referees themselves who accept their often laborious task. When such papers are read at a meeting of the Society, the paucity of the attendance and the lack of good discussion are obvious and distressing. In fact, it has been found well to take vast numbers of such papers as read, and even then the discussion of the few that are left is often disappointing.

It is on this account that greater stress has been laid recently on the provision of opportunities for organized discussions, in which some important subject of recent development is considered by the workers in that subject. The discussion ranges over a wider field than that of a single worker's account of his new discoveries. It is much more interesting and informing to the general body, as the very satisfactory attendances have shown. Clearly there is a desire to understand the main purpose and the principal achievements of each growing subject.

I believe that Fellows, and others, have welcomed the recent plan of publishing summaries of papers as an appendix to the *Proceedings* as soon as the papers are received, publication of the papers themselves being deferred until approval has been given in the usual way. Not merely is an earlier announcement of discovery made on this plan, but the collection of summaries presents the general trend of research to those who are not specialists

Summaries are the expression of a natural reaction to the difficulties caused by the growing specialization of research They represent a certain recognition of the principle that discovery must be accompanied by exposition, the principle that has been little regarded in past years, but now demands acceptance

The ideal summary is more than a mere digest or shortened form of the paper It differs from the paper itself in that it is addressed to a wider circle of readers which may include the experts but contains also many others who should, *in fact*, receive the principal attention For this reason it may be more difficult to write than the paper itself, because it must take into account the interests and the understandings of those who will rarely read the original paper, even if they could do so, but will gladly absorb the meaning and the information of the summary, especially if there is a connexion with their own work When the summary is written with this wider view it becomes literature, though the paper itself may be no more than a record

Another reason for greater attention to the writing of summaries is that specialization quickly takes explorers out of sight of each other, and out of sight of the main body It is natural for them to talk to each other only, and to forget everyone else Consequently, it becomes difficult to incorporate their work into any general scheme Also it is dangerous for researchers to be so far out of reach It is natural for them to lose a sense of proportion and to imagine, each one, that his is the main line, or at least a very important line of advance Who is to call a halt when this happens, unless the work that is going on is so far appreciated that a general opinion can be formed as to its advisability? It would, of course, be impossible to put the direction of all research into the hands of an elected committee or a dictator Yet a certain degree of control is required, and this is best exercised by general opinion reflected in the minds of those who direct in various ways the expenditure of money on research The only way to facilitate the growth of a general opinion which is sound, is to insist that a general account shall be given which is intelligible to a sufficient number

These considerations tempt me to ask whether it is necessary that our *Transactions* and *Proceedings* should contain so much as they do at present When a new departure in experiment or theory is made, or a new fact is discovered, or a new correlation, it is right that a careful and complete explanation should be given, and if this is not too long, it is excellent that it should appear in the *Proceedings* If the novelty leads, as it often does, to a steady output of observation, extension, confirmation and illustration, with many figures and bulky tables, there must come a time when complete publication to the world becomes unnecessary and even tiresome What the scientific world wants is a general account of progress made, so that its bearing may be clear A mass of details in continuation is of interest to other workers engaged in the same research, but very few others want the full account The *Proceedings* should contain papers of original discovery or fresh departure, as regards continuation papers, as they might be called, summaries properly written should be enough Continuation papers should, of course, be preserved and at least be available on demand, still better, a few hundred full-sized photograph copies could be made This could be done at a fraction of the cost of printing, especially if the printing is difficult, full of figures and symbols The *Proceedings* would then be much reduced in size, and would be more handy, interesting and useful

There is a further consideration of no small practical importance A largely attended conference on Documentation was held a few months ago at Oxford The subjects with which it dealt were of comparatively modern importance, but were clearly of the greatest interest in a number of places, particularly in patent offices, and in the libraries of industrial research associations The summaries of papers in scientific journals came in for much adverse comment, comment which seemed to be entirely just The point was that summaries were written for the expert only, and were often barely sufficient at that

Finally, if the scientific worker is no longer to be content merely to observe and to record, but also to share his knowledge with the world in general, he needs certain qualifications and a certain education which are much less necessary in the more restricted field He is dealing not only with facts but with men his work enters the humanities This is really a very great matter It draws its importance from the tremendous consequences of the increase of the knowledge of nature which are now obvious Lord Rayleigh has lately shown the absurdity of the charge that scientific men are responsible for the evil uses that are sometimes made of their discoveries But this responsibility is theirs, that they shall explain what they find so that their fellow-men know how they stand The scientist cannot be expected to see that discovery is rightly used, but no one except himself can rightly describe it It is not to be expected that the best use will be made of what is imperfectly understood

These are problems of tactics They cannot be fully solved while school and college still maintain an artificial division between two forms of teaching, naming one of them science and the other "humanities" They have far more in common than is generally recognized There must, of course, be specialization, a divergence in the later stages But the men of different types of mind must be kept together more than at present, so that when they meet in after-life they can understand each other It happens too often now that the administrator makes mistakes because he cannot understand his technician's advice, and the technician is incapable of expressing himself so that his administrator understands him There ought not, in fact, to be a sharp distinction between the two It is a fundamental point that humanities and science have joined hands in the service of mankind

Awards of Medals, 1938

Professor NIELS BOHR is awarded the COPLEY MEDAL He has been for many years the recognized leader of theoretical atomic physics His early work in 1913 provided the connecting link between Planck's conception of quanta and Rutherford's of the nucleus, and made it possible for the first time to constitute a consistent theory of spectroscopy The development of this theory owes nearly as much to Bohr as did its inception Among other things he invented the principle of correspondence according to which quantum phenomena pass over into the classical as a limiting case, according to certain definite rules which Bohr laid down This principle was of the greatest importance in the early days of the theory

Besides providing a rational explanation of atomic spectra, Bohr's theory made it possible to give a satisfactory explanation of the structure of atoms, and in particular to explain the changes in properties which occur as we go from one element to the next in Mendelejeff's periodic table In doing so it provided an explanation of the shells of electrons whose existence had been proposed by J J Thomson In the development of this theory Bohr's mathematical ingenuity and his application of the theory of perturbation played a large part

When the principles of wave mechanics were initiated by de Broglie and Heisenberg, the latter of whom was a pupil of Bohr's, Bohr took an important part in the development of the new ideas, and in transforming atomic theory to fit them The view which is held at the present day of the relationship between waves and particles is very largely due to Bohr

In the last few years Bohr has transformed the ideas held as to the structure of the nucleus by pointing out the intimate association of the particles which comprise it, and the necessity for treating them as part of a closely knit system more like a liquid than a gas This fundamental idea has already had far-reaching effects, and is the basis of almost all present work

Although his published papers are of supreme importance, it is probable that Bohr's greatest contribution to physics lies in the influence which he has exerted over an enormous proportion of the leading physicists of the world He possesses to an extraordinary extent the ability to draw ideas from minds which would otherwise probably never have produced them, and all who have fallen under his influence are conscious of his supreme power of inspiration

Professor ROBERT WILLIAMS WOOD is awarded the RUMFORD MEDAL The study of physical optics owes much to Professor Wood, who has been one of the leading experimenters in this field for the past 40 years There is hardly a branch of the subject which he has not enriched by the touch of his genius

Before the advent of Bohr's quantum theory, when our knowledge of the structure of atoms and molecules was very meagre, he had discovered the line and continuous absorption of sodium vapour, the phenomenon of resonance radiation of gases and vapours, and the quenching of this radiation by foreign gases These discoveries opened up rich fields of research and were of the greatest value to later workers in laying the foundations of the theory of atomic and molecular spectra

The elucidation of the phenomenon of resonance radiation demanded the utmost experimental skill and resource Nothing less powerful than an improvized 40 ft focus spectrograph sufficed for his work on the remarkable resonance spectra of molecules! Even now one cannot but admire the beautiful and ingenious experiments on the independent excitation of the yellow sodium lines

In addition to his researches on the resonance radiation of metallic and other vapours, Wood investigated their magnetic rotation and dispersion His work on the magneto-optics of sodium vapour both in the atomic and molecular state is now classical

More recent but belonging to the same domain of experiment are the very interesting discoveries of Wood and Ellett on the magneto-optics of resonance radiation

Wood's mastery of technique is universally acknowledged He has introduced many ingenious and striking devices to experimental method These are too numerous to catalogue here, but I would mention specially his method of the production of atomic hydrogen and his observations on the spontaneous incandescence of substances in atomic hydrogen which led to the invention of the atomic hydrogen welding torch by Langmuir, his very efficient and now widely used method of observing Raman Spectra, his échelette grating which has proved to be the grating *par excellence* for the investigation of the near and far infra-red, and his pioneer use of light filters in ultra-violet and infra-red photography

A ROYAL MEDAL is awarded to Dr FRANCIS WILLIAM ASTON Few cases are known to science in which an important development has remained so much in one man's hands as has the study of isotopes, by means of the mass spectrograph, in those of Dr Aston Aston's attention was drawn to isotopes by his work as assistant to Sir Joseph Thomson when the latter was working on the analysis of positive rays by the parabola method The results for neon suggested very clearly that this element contained atoms of weight 20 and 22, but other explanations were conceivable Aston attempted to settle this question by separating the two components Fractional distillation was carried out in an elaborate apparatus of his own devising and construction A microbalance was devised to measure the hoped-for change of density, but the result was negative Further attempts at separation by diffusion involving the construction of elaborate devices for repeating the diffusion process many thousand times gave inconclusive results As is now known, this was due to inadequate mixing After the war Aston started a new line of attack by improving the apparatus for the electromagnetic analysis of positive rays Known as the mass spectrograph, his instrument, which depends on an ingenious focusing of the rays, was powerful enough to show that the lighter constituent of neon had a mass less than corresponded to the density of the gas as a whole, which was therefore a mixture But the results went much further, and Aston found that not neon only but the majority of elements consisted of mixture of isotopes, in the sense already established by Soddy and others for certain products of radioactivity This discovery profoundly altered men's views as to the nature of the ordinary chemical elements For one thing, atomic weights as ordinarily determined were seen to be merely weighted means and not fundamental constants By various modifications of this method Aston was able to extend his experiments to cover the great majority of the known elements, and to establish the existence of hundreds of isotopes In addition he was able to show that, with the exception of hydrogen, all the isotopes had nearly integral atomic weights taking oxygen as 16 This result was of great importance in connexion with Rutherford's theory of the nucleus

The later developments of Aston's work are concerned mainly with the small deviations from this whole number law Since on the theory of relativity mass and energy are equivalent, the mass of a nucleus is a measure of its internal energy, and the small divergencies from whole numbers give the energy with which the constituent particles are bound together Using an improved mass-spectrograph capable of an accuracy of 1 in 20,000, Aston has determined this binding energy with considerable accuracy, at least for the lighter elements His figures have been checked in many cases by the results of atomic disintegrations, and, with similar measurements by Bainbridge, form an invaluable basis for the study of nuclear changes

By determining photometrically the proportions of the isotopes, Aston has been able to calculate "chemical" atomic weights, which in some cases have corrected those found by the older methods Thus both in chemistry and in physics Aston's work has been of outstanding value

A ROYAL MEDAL is awarded to Professor RONALD AYLMER FISHER His contributions, both to the development of the logical theory of statistical methods and to the invention of efficient tools for the use of the experimental worker, have been of outstanding importance Before Fisher entered the field (in 1912), the work of Galton, Pearson and their immediate pupils had widely extended the connotation of statistical methods, particularly in biological research Useful descriptive methods had been invented and reasonably adequate tests of sampling errors when samples were large (say, a hundred or more observations) were made available As a result there was an enormous increase in the number of workers who applied quantitative methods to biological, medical and sociological problems It may be said that in freeing the statistical methods of weaknesses obvious a quarter of a century ago, Fisher has been one of the chief contributors to the improvement of the logical basis of statistical methodology He has been the most important contributor both to the theory and practice of small sample analysis, and to the armamentarium of statistical tools for biological and technological research His own practical contributions to the study of genetics have been neither few nor unimportant (his treatise *The Genetical Theory of Natural Selection*, 1930, and papers in the B *Proceedings* and *Transactions* of the Society are examples), but the scope of his work has covered a much wider field of scientific research interesting both the physical and biological sides of the Society

The DAVY MEDAL is awarded to Professor GEORGE BARGER He has been continuously engaged in research, mainly on the constitution and synthesis of natural products, since 1902

His two most important contributions to knowledge are linked together, namely, his work on the alkaloids of ergot and on the sympathomimetic amines

After his isolation (with Carr) of the alkaloid ergotoxine, to which the effect of ergot on the cock's comb is due, he showed that the pressor bases from putrid meat were the products of decarboxylation of amino-acids, and included *p*-hydroxyphenylethylamine derived in this way from tyrosine He worked out methods for the synthesis of *p*-hydroxyphenylethylamine and allied compounds, and proved that this compound and also histamine, the product of decarboxylation of histidine, occurred in extracts of ergot

One of the earliest systematic investigations of the relation between chemical constitution and pharmacological properties was the work which he carried out in collaboration with Dale on the sympathomimetic amines of the phenylethylamine class

He has made considerable contributions to our knowledge of the constitution of many alkaloids, including carpaine, physostigmine and yohimbine, and also of many naturally occurring derivatives of amino-acids, such as carnosine, ergothioneine and methionine Special mention may be made of his work, carried out jointly with Harington, on the constitution and synthesis of thyroxine

The DARWIN MEDAL is awarded to Professor FREDERICK ORPEN BOWER His main contributions to botany have been the intensive study of the morphology and affinities of ferns and allied plants A series of papers published in the *Transactions* of the Royal Society and the *Annals of Botany* on the development of spore-bearing organs in the Pteridophyta is a contribution to phylogeny of great importance In his book *Origin of a Land Flora* (1908) Bower discussed evolutionary problems with special reference to the Bryophyta and Pteridophyta 26 years later he dealt with the same problem in *Primitive Land Plants* His three volumes on ferns (1923–8) are a classic work on the affinities of members of this group and a most valuable source of information

In 1930 he discussed *Size and Form in Plants* His latest book, *Sixty years of botany in Britain*, written in his eighty-third year, is in part autobiographical Professor Bower has consistently devoted himself to a branch of botanical science in which he has long been regarded as a

leading authority By his own researches and the judicial examination of recorded facts he has thrown light on the natural affinities of certain groups of plants

The HUGHES MEDAL is awarded jointly to Dr JOHN DOUGLAS COCKCROFT and Dr ERNEST THOMAS SINTON WALTON The discovery by Dr Cockcroft and Dr Walton that the transmutation of elements can be effected by means of artificially accelerated particles has opened up a new line of work of outstanding interest and importance In previous experiments, initiated by Rutherford as long ago as 1919, the transmutation of several elements had been produced by bombarding them with the α-particles from the natural radioactive substances In this early work it was thought that bombarding particles of very great energy were required to disrupt atomic nuclei The development of quantum mechanical ideas to nuclear problems made it possible for the first time to see how charged particles of low energy could penetrate into an atomic nucleus, and, moreover, enabled calculations to be made of the probability of penetration It was on the basis of such calculations that Cockcroft and Walton came to the conclusion that protons accelerated by moderate potentials, of the order of a few hundred kilovolts, should be able to effect the disintegration of the lighter elements During 1930 attempts were made to disintegrate lithium by bombardment with protons using accelerating voltages up to 300,000 V The results were at first negative but continued experiment, in which the apparatus was redesigned to give voltages up to 600 kV, was in 1932 rewarded with the successful disintegration of lithium by bombardment with protons Thus for the first time an atomic transmutation was effected by means entirely under the experimenter's control

These experiments of Cockcroft and Walton gave a tremendous impetus to investigations in nuclear physics Their work has been taken up in many laboratories and in many countries, and it has given rise to such a mass of experimental results as to form almost a new branch of science

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A quantitative investigation into the enterohepatic circulation of bile salts in the cat

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In 1870 Schiff put forward his hypothesis of the enterohepatic circulation of the bile He observed that if a large fistulous opening be made in dogs between the surface of the abdomen and the gall bladder without the common bile duct being ligatured, the bile flows externally If, however, the fistulous opening is closed, then the bile flows into the intestine Using dogs with "amphibolic" fistulae Schiff found that the quantity of bile obtained from such fistulae was much greater immediately after the bile had been allowed to flow into the intestine than when it had been flowing externally The part played by bile salts in this phenomenon he established by the introduction of bile salts into the intestine through a duodenal fistula This procedure increased both the quantity of bile and the amount of bile salts secreted The circulation of the bile salts has been confirmed by many observers Weiss (1884) fed glycocholic acid to dogs and found the corresponding acid in the bile subsequently excreted although dog's bile normally contains taurocholic acid only

There are differences of opinion in regard to the circulation of bile pigments Whipple (1922), as the result of an extensive series of investigations, came to the conclusion that there is no evidence for the circulation of bile pigments On the other hand Brown, McMaster and Rous (1923) fed sheep's bile which contains a characteristic pigment, cholohaematin, to dogs and found it in fistula bile Further, they observed that dogs fed on dog bile often showed a marked increase in the output of bilirubin in the bile subsequently excreted Similarly, McMaster and Elman (1925) found that pure bilirubin given by the mouth or injected into the duodenum causes an increase in the bilirubin content of fistula bile

In this paper the quantitative phenomena observed in the secretion of bile from the liver under varying conditions are described Continuous samples of secreted bile have been analysed and their cholic acid contents determined From these data precise information has been obtained on the enterohepatic circulation of the bile salts

1 METHODS

(*a*) *Operative technique* The majority of the experiments were carried out on cats Anaesthesia was maintained by the intravenous injection of chloralose (0 08 g /kg) after preliminary induction with ether or ethyl chloride Tracheotomy was performed and intravenous injections were made through a cannula inserted into the right saphenous vein The abdomen was opened by a mid-line incision, and the gall bladder was emptied after a ligature had been tied round its neck A cannula was inserted into the common bile duct near its entrance into the duodenum, and, in some experiments in which the pancreatic juice was collected, a second cannula was tied into the pancreatic duct The liver bile was collected and the amounts secreted during each hour recorded These hourly samples were assayed for cholic acid

(*b*) *Estimation of cholic acid* The cholic acid content of the bile samples was estimated colorimetrically The pigment was first removed by the method of L D Scott (1933) 0 4 c c of bile secreted directly from the liver, or 0 2 c c of gall bladder bile, were added to about 10 c c of absolute alcohol and boiled for 3 min in a water bath After cooling, absolute alcohol was added to bring the volume to 20 c c and the tarry precipitate was filtered off To 15 c c of the filtrate 1 c c of saturated aqueous barium hydroxide was added and the mixture boiled gently for 3 min , during which time a considerable amount of flocculent precipitate formed The suspension was then allowed to stand in a refrigerator for not less than 30 min As more complete precipitation of the pigments occurred if the tubes were kept cold for a longer period, they were usually kept in the refrigerator overnight The precipitate was then filtered off and the colourless filtrate was assayed for cholic acid, 1 c c of the filtrate containing 3/64ths of the total acid in the original volume of the sample taken

The cholic acid content of the filtrates was estimated by the blue colour reaction of Gregory and Pascoe (1929)

A series of standard tubes were set up containing 0 1, 0 2, , 0 9 and 1 c c of 0 1 % cholic acid in absolute alcohol Tubes containing 0 5, 2 0 and 8 0 c c of the filtrate from the sample were also prepared All the tubes were placed in a hot water bath and evaporated to dryness To each tube 1 c c of freshly prepared 0 15 % aqueous furfural and 3 c c 45 % H_2SO_4

were added After thorough mixing of the contents the tubes were immersed in a water bath at 65° C for 30 min The blue colour of the samples was matched immediately against the standard tubes

By using this range of standards and this wide range of quantities for the samples a large variation of concentration of bile acid in the original specimens of bile allowed at least one tube of the three to be matched against a standard Thus, samples of bile containing as much as 10 % or as little as 0 07 % cholic acid could be estimated without modification of the method

Further, the method allows samples of bile containing as little as 0 2 mg of cholic acid to be assayed accurately It was therefore possible to carry out a series of analyses on small successive quantities of bile, collected hourly, during an acute experiment and thereby to determine the origin or fate of bile salt under a definite experimental procedure

2 THE AMOUNT OF CHOLIC ACID IN THE BILE OF THE FASTING AND FED CAT

Many investigators have determined the effect of various procedures on the biliary apparatus of the liver by measuring the volume of bile produced in definite periods of time In point of fact, however, the bile salts confer on bile the physiological properties which determine its importance as a digestive secretion Although it is generally true that an increased secretion of bile salt is accompanied by an increased volume of bile yet under many experimental conditions the volume of bile may show no change with large variations in the bile salt content In the account of these experimental results, therefore, although the volume of bile secreted is usually recorded, yet greater attention has been paid to the absolute amount of bile salt secreted (estimated as cholic acid) under definite conditions

(*a*) *The fasting cat* Although the secretion of bile is a continuous process yet the rate of secretion is greatly modified by the state of digestion A cat which has not been fed for 24 hr usually secretes from 0 5 to 1 c c of bile per hour The volume of bile secreted is fairly constant but the cholic acid content diminishes to a very small amount as secretion proceeds The following figures illustrate typical experimental results obtained from a cat which had not been fed for 20 hr

The bile obtained from the gall bladder at the beginning of the experiment amounted to 3 c c and contained 9 6 % cholic acid The cholic acid content of this bile was therefore 288 mg

The bile secreted from the liver during the next 6 hr gave the following figures

Time hr	Vol (c c) of bile per hr	Cholic acid (mg) per hr
1	0 3	8 7
2	0 4	4 3
3	0 6	2 0
4	0 5	0 6
5	0 5	0 5
6	0 5	0 5
Total	2 8	16 6

The results show that in the fasting cat almost the whole of the bile salt is contained in the gall bladder bile, whilst the amount contained in the continuously secreted hepatic bile is of very small dimensions The low bile salt content of the liver bile in the fasting cat differs from that described for animals with permanent biliary fistulae Thus Bidder and Schmidt (1852) found that the flow of bile from fistulae in fasting cats greatly diminished in volume but the percentage of solids in the bile was maintained Okada (1915), on the other hand, found the biliary secretion diminished both in volume and in total solids during starvation The most striking results, however, were obtained by Foster, Hooper and Whipple (1919) who found that a fistulous dog excretes considerable quantities of bile acid during fasting periods They assumed that under these circumstances the bile acid excreted represented the rate of synthesis in the fasting fistulous animal The figures given above show that in an acute experiment on a fasting anaesthetized cat the rate of synthesis of cholic acid is less than 0 5 mg /hr In a subsequent section experimental results are described on unanaesthetized fed cats which indicate that re-formation of cholic acid in an animal deprived of the greater part of its bile may take place at the rate of 100 mg /day or approximately 4 mg /hr This figure is about eight times that of the rate of excretion of cholic acid in the fasting anaesthetized cat and may indicate either the effect of diet on the synthesis of bile salt or the depressant action of anaesthetics on the bile salt mechanism

(*b*) *The fed cat* The following results were obtained from a cat which had been well fed 6 hr previous to the start of the experiment The animal was in an active state of digestion

The gall bladder contained only 1 3 c c of bile and the cholic acid content was 1 07 %, the gall bladder therefore contained 13 9 mg cholic acid

The bile secreted from the liver during the next 7 hr was collected and assayed for cholic acid The figures shown on next page were obtained

Three facts are shown by these figures (1) the small volume of bile and its low bile salt content in the gall bladder of the cat in active digestion,

(2) the relatively large quantity of bile salt secreted in the hepatic bile, and (3) the increase in volume of the hepatic bile

It is evident, however, that a great part of the bile salt of this cat was contained in some part of the extra hepatic system since only 87 mg of cholic acid can be accounted for in the gall bladder bile and hepatic bile, whereas a cat weighing 3 kg contains on the average 300 mg of cholic acid

Time hr	Vol (c c) of bile per hr	Cholic acid (mg) per hr
1	1 2	7 2
2	1 8	10 8
3	1 5	7 0
4	1 3	7 8
5	1 1	9 6
6	0 9	14 4
7	1 0	16 0
Total	8 8 c c	72 8 mg

(*c*) *The fed cat with its small intestine removed* In this experiment the effect of removal of the small intestine from a fed cat on the cholic acid content of the hepatic bile was determined

The cat was fed 6 hr before the start of the experiment as in the previous case The bile secreted for an initial period of 2 hr was collected and its cholic acid content determined At the end of this time the small intestine was removed The volume of bile and its cholic acid content was determined hourly for the next 5 hr

Time hr	Vol (c c) of bile per hr	Cholic acid (mg) per hr
1	2 8	74 8
2	2 7	25 9
	Small intestine removed	
3	2 5	5 0
4	1 6	1 6
5	1 3	0 9
6	1 3	0 65
7	1 0	0 5

In this animal the gall bladder was practically empty There was a very large amount of cholic acid (100 mg) contained in the hepatic bile secreted during the initial 2 hr period The removal of the small intestine, however, at once caused the cholic acid content of the hepatic bile to drop to the level of the fasting cat indicating that the immediate source of the cholic acid secreted in the hepatic bile was the bile salt absorbed from the small intestine The results gave a striking demonstration of the part played by the small intestine in the enterohepatic circulation of the bile salts

3. THE ABSORPTION OF BILE SALT FROM DIFFERENT PARTS OF THE ALIMENTARY CANAL

The foregoing experimental results suggested a method for determining the rate of absorption of bile salt from different segments of the alimentary canal. The principle of the method was to determine the rate of secretion of cholic acid in the hepatic bile of a fasting cat for 2 hr. Then dilute bile was put into that part of the alimentary canal it was desired to investigate. The effect on the cholic acid content of the subsequently secreted bile showed the degree to which absorption had taken place.

(*a*) *Duodenum and jejunum.* Cannulae were inserted into the common bile duct and pancreatic duct of a fasting cat after the gall bladder had been ligatured at the neck. The bile was removed from the gall bladder and diluted to 25 c.c. with 0.15% $NaHCO_3$. The hepatic bile was collected for 2 hr. and then the dilute bile was injected into the duodenum and jejunum isolated between two ligatures placed at the pyloric sphincter and upper end of the ileum. The secretions of bile and pancreatic juice were collected over a period of 7 hr. The following results were obtained:

Time hr.	Vol. (c.c.) of bile per hr.	Cholic acid (mg.) per hr.	Pancreatic juice (c.c.) per hr.
1	2.6	9.1	0
2	3.3	4.1	0
Dilute bile injected into the duodenum and jejunum			
3	2.7	3.5	5.0
4	3.5	4.5	7.7
5	2.9	5.2	7.1
6	3.0	10.8	4.0
7	2.5	6.7	2.2

The most spectacular effect was the large secretion of pancreatic juice (26 c.c.) in the 5 hr. following the injection of the dilute bile into the small intestine, an effect due to the absorbed bile salt carrying the secretin contained in the duodenal mucosa into the blood (Mellanby 1926). It is evident from the cholic acid content of the bile that although bile salts are absorbed from this part of the small intestine yet absorption is relatively slow. In the fasting cat the average secretion of bile salt during this 5 hr. contains 5–10 mg. of cholic acid. In this cat the absorption of the dilute bile from the duodeno-jejunal segment raised the cholic acid content of the hepatic bile to 30.7 mg., rather less than one-third of the cholic acid content of the injected bile (107 mg.).

(*b*) *The ileum.* In these experiments gall bladder bile diluted with

0 15 % $NaCHO_3$ was injected into the ileum, isolated between two ligatures, one placed at the lower end of the jejunum and the other at the ileo-colic sphincter

Time hr	Vol (c c) of bile per hr	Cholic acid (mg) per hr
1	1 1	17 6
2	1 9	10 8

Cat's gall bladder bile (3 c c), diluted to 25 c c with 0 15 % $NaHCO_3$, injected into the ileum

Time hr	Vol (c c) of bile per hr	Cholic acid (mg) per hr
3	2 2	8 8
4	2 5	13 0
5	2 6	20 8
6	2 7	28 9
7	2 8	22 4

It is evident that bile salts are absorbed much more rapidly from the ileum than from the upper part of the small intestine—the volume of liver bile secreted per hour was almost doubled and the amount of cholic acid contained in the 5 hr secretion rose to 94 mg No pancreatic juice was secreted throughout the experiment a fact which agrees with the observation that no secretin is contained in the mucous membrane of the ileum of the cat (Mellanby and Huggett 1926)

(*c*) *The large intestine and rectum* General considerations indicate that in the cat digestion and absorption are fairly complete before the contents of the alimentary canal pass into the large intestine In accordance with this assumption the following experimental results show that absorption of bile salts from the large intestine is slow and incomplete

Dilute cat's bile (40 c c containing 0 27 % cholic acid) was injected into the large intestine of a fasting cat, the intestine having been first emptied and washed out with Ringer and ligatured at the ileo-colic sphincter and the internal sphincter of the anus

The hourly secretion of cholic acid in the hepatic bile before the intestinal injection of dilute bile was 27 7 mg /hr In the succeeding 5 hr the average secretion of cholic acid fell to 8 1 mg /hr , a figure which is above that of the fasting cat (average about 2 mg /hr) The amount of fluid removed from the large intestine after the experiment was 4 5 c c containing 1 46 % cholic acid This fact is remarkable since the injected bile contained only 0 27 % cholic acid, i e there had been a differential absorption of water from the large intestine so that the residual bile salt concentration increased fivefold

The mucous membrane of the large intestine was extracted with alcohol This extract contained 28 mg of cholic acid

From these figures it is possible to account for the greater part of the

108 mg of cholic acid contained in the dilute bile injected into the large intestine Thus there remained in the lumen of the gut 4 5 c c of fluid containing 1 46% cholic acid or 65 7 mg of cholic acid A further 28 mg of cholic acid were extracted from the intestinal mucosa by alcohol Therefore there remains only 15 mg of cholic acid to be accounted for and this may be taken as the increased secretion of cholic acid in the hepatic bile beyond that secreted by the normal fasting cat

It is evident that bile salts are absorbed with difficulty from the large intestine and that any fluid containing them tends to concentrate the bile salt within it owing to the preferential absorption of water Further, it appears that the rapid passage of the contents of the small intestine into the large intestine may involve a considerable loss of bile salts to the animal

4 THE RATE OF EXCRETION OF BILE SALTS FROM THE BLOOD

The presence of recognizable quantities of bile salt in the blood is disputed Some observers give values ranging from 3 to 6 mg of bile salt in 100 c c of normal blood (Aldrich and Bledsoe 1928, Charlet 1929) On the other hand Gregory and Pascoe (1929) and Walker (1930) state that demonstrable quantities of bile salts do not exist in normal blood Many of the estimations are based on a modified Pettenkofer reaction Walker has shown that such a reaction is given by cholesteryl oleate and this compound is present in the blood of the ox, dog, horse, rabbit and man The absence of bile salts from the blood forms the basis of an experiment to determine whether these salts are stored in any tissue of the body outside the enterohepatic apparatus

The initial secretion of cholic acid in the bile of a fasting cat was at the rate of 4 9 mg /hr

112 mg of cholic acid dissolved in 12 c c alkaline Ringer was divided into 12 equal portions and 1 c c was injected every 15 min into the right saphenous vein During the next 3 hr the secretion of cholic acid in the hepatic bile rose to 30 7, 39 3 and 27 9 mg respectively and in the hour succeeding the injections fell to 9 5 mg The total amount of cholic acid injected was 112 mg , within an hour of the last injection 107 mg had been secreted in the hepatic bile

The results give a striking demonstration of the non-threshold character of bile salts in regard to the secretory cells of the biliary apparatus of the liver They also indicate that bile salts are not stored in any tissue of the cat outside the enterohepatic system

5 SECRETIN AS A CHOLAGOGUE

In 1902 Bayliss and Starling showed that the intravenous injection of a solution of secretin, free from bile salts, into a dog increased the flow of bile Since that time the cholagogue action of secretin has been repeatedly confirmed

A quantitative series of experiments were carried out to determine whether the increased volume of bile secreted under the influence of intravenous secretin injections was accompanied by an increased secretion of bile salts The volume of bile secreted per hour and its cholic acid content were determined in a fasting cat over a period of 2 hr During the next 3 hr 0 6 mg of secretin dissolved in 6 c c of 0 1 % $NaHCO_3$ was injected in quantities of 1 c c at half-hourly intervals into the right saphenous vein During this time the bile and pancreatic juice were collected and after the cessation of the secretin injections the normal flow of bile was observed for a further period of 2 hr

Time hr	Vol (c c) of bile per hr	Cholic acid (mg) per hr	Pancreatic juice (c c) per hr
1	2 0	22 6	0
2	2 4	9 6	0
0 6 mg secretin injected intravenously in quantities of 0 1 mg every half hour			
3	3 4	4 7	2 8
4	3 5	2 4	3 0
5	3 1	1 2	3 3
6	2 5	1 2	0
7	2 1	0 6	0

The well known effect of secretin in augmenting the volume of bile is evident from the figures The average quantity of bile produced per hour before and after the secretin injections was 2 2 and 2 3 c c respectively During the secretin injections the volume rose to 3 3 c c A 50 % increase in volume of the bile secreted is the change usually observed during secretin injections On the other hand, the total quantity of cholic acid contained in the bile decreased steadily during the secretin injections There was no indication that secretin increases the secretion of cholic acid, the essential constituent of the bile viewed from the standpoint of digestion It is an interesting fact that secretin exerts the same type of action on the external secretion of the pancreas and on the secretion of bile In the former case secretin determines the volume of dilute $NaHCO_3$ in which the pancreatic enzymes are contained, the enzyme formation being a function of the vagus nerve, and in the case of the bile secretin determines the volume of fluid but has no influence on the essential cholic acid content

6 THE INFLUENCE OF THE AUTONOMIC NERVES ON THE SECRETION OF BILE

In 1869 Pfluger observed that section of the nerves supplying the liver had no influence on the secretion of bile In 1902 Langley investigated the effect of the sympathetic system on bile secretion by using an aqueous extract of suprarenal gland dried *in vacuo* He found that such an extract caused a temporary increase followed by a decrease of hepatic secretion If, however, the gall bladder was not tied off there was invariably a diminution of the bile flow This he assumed to be due to inhibition of the gall bladder Other observers (Downs and Eddy 1919, Adachi 1923, Specht 1933) have described a reduction of bile flow after the injection of adrenalin Although it is generally agreed that sympathetic stimulation diminishes the volume of bile, yet Neubauer (1923) observed, in rabbits, an increase of bile solids after stimulation of the splanchnic nerves In regard to the parasympathetic nerves to the liver Adachi (1923) found that acetylcholine retarded the outflow of bile in fistulous dogs On the other hand, Brugsch and Horsters (1926) found that parasympathetic stimulation by means of pilocarpine and acetylcholine caused an increase of bile flow

In these experiments precautions were taken to exclude effects due to changes in the absorption of bile salts from the alimentary canal The experiments were therefore carried out on fasting cats Again precautions were taken to exclude all extraneous effects due to the musculature of the gall bladder and its ducts The gall bladder was emptied and the cystic duct was tied as near as possible to the common bile duct

(*a*) *The parasympathetic nerves* Paralysis of these nerves in a fasting cat was produced by the intravenous injection of 5 mg of atropine The atropine was injected after the normal hepatic secretion had been collected for 2 hr

Time hr	Vol (c c) of bile per hr	Cholic acid (mg) per hr
1	0.6	12.0
2	0.5	6.0
	5 mg atropine injected	
3	0.8	4.2
4	0.7	1.6
5	0.75	1.4
6	0.6	1.1
7	0.6	0.7

The excretion of bile and its cholic acid content before and after the injection of the atropine is the same as that observed in the normal fasting cat. There is no evidence that general parasympathetic paralysis influences the volume of bile secreted or its cholic acid content.

A second series of experiments were carried out in which general parasympathetic stimulation was produced by the subcutaneous injection of acetylcholine (3 mg.) into an eserinized cat (1 mg.). The normal hepatic secretion was collected for 2 hr. before the injection of the eserine and acetylcholine. The following results were obtained:

Time hr.	Vol. (c.c.) of bile per hr.	% of cholic acid	Cholic acid (mg.) per hr.
1	3.0	0.67	20.1
2	3.9	0.17	6.6
	Eserine and acetylcholine injected		
3	2.7	0.10	2.7
4	3.1	0.10	3.1
5	2.9	0.10	2.9
6	2.6	0.07	1.8
7	2.4	0.07	1.7

Eserine (1 mg.) followed, after 15 min., by acetylcholine (3 mg.) was injected. Powerful stimulation of the parasympathetic system was evident from the large secretion of saliva.

The results show that parasympathetic stimulation produced by acetylcholine has no effect on the volume of bile secreted nor on the quantity of cholic acid contained in it. From the results of these experiments with atropine and acetylcholine it is clear that the vagus nerve does not influence the biliary hepato-secretory apparatus in the cat.

(*b*) *The sympathetic nerves.* Stimulation of the sympathetic system in a fasting cat was obtained by the intravenous injection of small quantities of adrenalin. The following figures show the results obtained by the intravenous injection of 2 mg. of adrenalin divided into 12 equal portions, injected at equal intervals of time (10 min.), during a period of 2 hr. The bile was collected for 2 hr. before, during, and 3 hr. after the adrenalin injections. The figures show a small diminution of bile secretion during the adrenalin injections and confirm the general results of earlier workers. A striking effect, however, is the continued fall in bile flow for 3 hr. after the cessation of the intravenous injections of adrenalin.

The figures showing the cholic acid content of the bile are of interest. There is no continuous fall of cholic acid content comparable to that seen in the normal fasting cat, in fact, during the adrenalin injections the total cholic acid excretion kept up at a relatively high level while in the succeeding 3 hr there was a large increase in the cholic acid content of the bile which more than compensated for the diminished volume The results indicate that under the influence of general sympathetic stimulation by adrenalin there is a synthesis of cholic acid from some precursor

Time hr	Vol (c c) of bile per hr	% of cholic acid	Cholic acid (mg) per hr
1	1 0	2 13	21 3
2	1 1	0 93	10 2
2 mg of adrenalin in 12 equal portions injected into the saphenous vein at 10 min intervals			
3	0 9	0 67	6 0
4	0 8	0 67	5 4
5	0 7	0 67	4 7
6	0 6	0 80	4 8
7	0 6	1 60	9 6

The synthesis of bile salt under the influence of general sympathetic stimulation (adrenalin) and the absence of effect of parasympathetic stimulation (acetylcholine) and paralysis (atropine) is the reverse of that observed with the external secretions of the gastric mucosa and pancreas

7 THE TOTAL AMOUNT OF CHOLIC ACID IN THE CAT AND RAT

(*a*) *The cat* The total amount of cholic acid in the enterohepatic system of a cat can be readily determined After 24 hr alimentary rest the greater part of the cholic acid is contained in the gall bladder bile This bile is therefore removed and its cholic acid content determined The remaining cholic acid of the animal is secreted in the bile collected from the liver over a period of 7 hr After about 4 hr the hepatic secretion in the fasting cat usually contains only about 1 mg /c c of bile A simple calculation gives an approximate value for the total cholic acid content of the animal

The following figures give the result of a typical experiment

Time hr	Vol (c c) of bile per hr	% of cholic acid	Cholic acid (mg) per hr
1	1 2	1 87	22 4
2	1 2	0 53	6 4
3	1 1	0 27	3 0
4	1 1	0 13	1 4
5	0 9	0 13	1 2
6	0 9	0 13	1 2
	Total 6 4		35 6

The gall bladder contained 4 2 c c of bile which assayed 4 27 % of cholic acid This bile therefore contained 179 3 mg cholic acid

The bile was collected from the liver during a period of 6 hr and assayed for cholic acid

The cat (weighing 2 9 kg) contained 179 3 mg of cholic acid in the gall bladder bile and 35 6 mg was secreted by the hepatic bile giving a total of 215 mg cholic acid in the animal

(*b*) *The rat* The rat possesses no gall bladder Therefore the bile is stored in the capacious bile ducts or is continuously circulating in the enterohepatic system It was of interest, therefore, to compare the cholic acid content of this type of animal with the cat

The following figures show the hourly secretion of bile and its cholic acid content in a fasting rat weighing 300 g

Time hr	Vol (c c) of bile per hr	% of cholic acid	Cholic acid (mg) per hr
1	0 7	0 45	3 15
2	0 5	0 21	1 0
3	0 7	0 11	0 8
4	0 6	0 11	0 65
5	0 45	0 12	0 5
6	0 7	0 04	0 3
7	0 5	0 10	0 5
	Total 4 15		6 9

This rat therefore contained only about 7 mg of cholic acid in its enterohepatic circulation

The cat of a weight approximately ten times that of the rat contained 215 mg cholic acid Therefore the cat contained three times as much cholic acid per unit of weight as the rat

It may be observed, however, that in the rat the average volume of bile secreted per hour was 0 6 c c and in the cat 1 c c /hr Therefore, for unit weight, the average volume of bile secreted by the rat is six times that of the cat, a figure which more than compensates for the relative cholic acid ratios of the cat and rat

8 THE RATE OF FORMATION OF CHOLIC ACID

Bidder and Schmidt (1852) calculated that seven-eighths of the total quantity of bile entering the intestine is reabsorbed Jenke (1932), by estimations of the bile acid content of fistula bile and the amount of bile acid in the faeces, calculated that the daily loss was one-seventh of the total bile acid contained in the animal It was a matter of interest therefore to determine the rate of synthesis of cholic acid in the cat Owing to the fact that almost all the bile salt is stored in the gall bladder of a fasting cat the information was readily obtained

A fasting cat was anaesthetized with ether and operated on under aseptic conditions * The gall bladder was exposed, and the bile removed from it by a syringe The site of puncture was ligatured and the abdominal opening closed

The animal quickly recovered from the operation and within 6 hr was eating fish and drinking milk

After 2 days the total cholic acid in the cat was determined by a complete experiment in which the bile was taken from the gall bladder and the hepatic bile collected over a period of 6 hr

The total quantity of cholic acid was determined in the various specimens of bile The following figures summarize the results 3 04 of bile containing 8 5 % cholic acid was removed at the operation from the gall bladder, that is, 255 mg of cholic acid

In the complete experiment 2 days later the gall bladder contained 1 8 c c of bile assaying 8 % cholic acid—162 6 mg cholic acid

The hepatic bile secreted during a period of 6 hr contained 46 2 mg cholic acid Therefore the total cholic acid produced in 2 days was approximately 209 mg after the removal of 255 mg

The results indicate that there is a considerable capacity for synthesis of bile salt in the cat, much greater than required for the replacement of a daily loss of 15 % in the faeces

It is evident, however, that the rate of synthesis of bile salt is largely determined by the nutritional state of the animal The cat from which the above figures were obtained suffered no apparent ill effects from the operation and ate well immediately after recovering from the anaesthetic Another cat, although operated on in precisely the same way, refused to eat The cholic acid assays in this cat gave the following results 2 c c of bile containing 6 1 % cholic acid were removed from the gall bladder at the operation—that is 122 mg cholic acid

Four days later the cat was anaesthetized and the total cholic acid determined

The gall bladder contained 0 2 c c of bile which had a content of 21 mg of cholic acid, and no bile was secreted by the liver

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* The aseptic operations were carried out for us by Dr C L G Pratt, to whom we desire to record our thanks

The authors desire to record their thanks to Messrs Sandoz, Basle, for their gift of pure bile acids used in these experiments

SUMMARY

1 In a fasting cat about 95 % of the total bile salt is contained in the gall bladder bile The hepatic bile, continuously secreted by the liver, contains only traces of bile salt

2 In the fed cat the volume and bile salt content of the hepatic bile shows a large increase whilst the gall bladder contains only a small quantity of relatively dilute bile The removal of the small intestine causes an immediate fall in the cholic acid content of the hepatic bile to that found in the fasting cat

3 Bile salts are absorbed more rapidly from the ileum than from the duodenum and jejunum A small amount of absorption occurs in the large intestine but the absorption of water takes place much more rapidly than that of bile salt Consequently, the original concentration of bile salt may be increased fivefold in the unabsorbed bile

4 Bile salts injected into the blood are rapidly excreted in the hepatic bile They are non-threshold substances in respect of the biliary apparatus Bile salts are not stored in any tissue outside the enterohepatic system

5 The intravenous injection of secretin increases by 50 % the volume of bile secreted by the liver It has no effect on the total quantity of cholic acid contained in the bile Its action is therefore that of a hydrogogue

6 Stimulation (acetylcholine) or paralysis (atropine) of the parasympathetic nerves to the liver has no influence on the volume or cholic acid content of the bile

7 Stimulation of the sympathetic nerves to the liver (adrenalin) diminishes to a small degree the volume of the bile secreted but augments the total amount of cholic acid contained in it The sympathetic nerves may stimulate the formation of cholic acid from some inactive precursor by the liver cells

8 After removal of the bile from a fasting cat, two-thirds of the cholic acid is resynthesized in 2 days if the animal feeds well In the absence of eating no synthesis of cholic acid takes place

9 A cat contains approximately 100 mg of cholic acid per kg of body weight A rat contains approximately 25 mg cholic acid per kg of body weight The rate of flow of bile per unit of body weight is about six times as great in the rat as the cat

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Haemocuprein and hepatocuprein, copper-protein compounds of blood and liver in mammals

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[Plate 11]

Introduction

The occurrence of copper in plant and animal tissues has been known for more than a century Buchholtz (1816) and Meissner (1817) reported its presence in plants, Boutigny (1833) found it in animal tissues, and its presence in tissues of man was discovered by Devergie and Hervy (1838) and Devergie (1840)

That copper is present in human blood has been recognized since the work of Deschamps (1849), and this fact was, later, generally accepted (Porter 1875) Its concentrations in the red blood corpuscles and serum of man and different animals of different ages, under normal and pathological conditions, have been estimated by several workers As to the distribution of copper between red blood corpuscles and serum, the statements are still somewhat contradictory

While Elvehjem, Steenbock and Hart (1929) obtained higher values for red blood corpuscles, Schindel (1931) and Guillemet (1932) found, on the contrary, more copper in plasma

The problem as to the state in which copper occurs in blood was approached by several workers but so far all attempts to determine it were carried out only on the copper found in serum

The supposition that in serum the copper is present in a more or less loose combination with an organic compound, probably a protein, was supported by several observations Thus, it was found that the copper cannot be removed from serum by dialysis (Abderhalden and Moller 1928), but it can be very easily liberated from its loose compound by weak hydrochloric acid (Warburg and Krebs 1927) or by trichloroacetic acid (Locke and others

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1932) and that by progressively lowering the pH of serum, an increasing amount of copper dialyses out (Boyden and Potter 1937) It was shown by Tompsett (1934) that even the copper of the whole blood can be completely extracted with trichloroacetic acid Moreover, the results of cataphoresis experiments by Eisler, Rosdahl and Theorell (1936) suggest that in horse serum copper is present as a compound with albumin

Although these observations support the view that the copper in blood is present as a compound with a protein, no attempt has been made to isolate this compound in a purified state in order to ascertain its true nature

In this paper we describe a simple method of isolation from red blood corpuscles and from horse serum of a copper-protein compound (*haemocuprein*) in a pure crystalline state and compare it with another copper-protein compound (*hepatocuprein*), which we have obtained in highly purified state from mammalian livers

METHODS OF COPPER ESTIMATION

Throughout this investigation the copper was estimated colorimetrically using diethyl-dithio-carbamate Two different procedures have been adopted for the combustion of the material

(1) In all the preparations except the blood corpuscles, the material containing from 0 005 to 0 03 mg Cu is placed in a Kjeldahl flask, evaporated almost to dryness and destroyed with 2 c c sulphuric acid by heating it over a small flame When the combustion is complete the fluid is diluted with 20 c c glass distilled water, neutralized, mixed with 5 c c 40 % citric acid solution and made alkaline with ammonia The mixture is quantitatively transferred to a separating funnel to which is added 10 c c of 0 1 % solution of sodium diethyl dithio carbamate and extracted with four lots (3 c c each) of carbon tetrachloride The combined yellow extracts are transferred to a small cylinder of the Wolff colorimeter and its colour compared with that of a carbon tetrachloride extract of a standard copper solution treated with sodium diethyl dithio carbamate solution

(2) For the estimation of copper in blood corpuscles, the latter, after a thorough washing with salt solution, are plasmolysed with an equal volume of distilled water The solution, evaporated to dryness, is ashed for 5 hr in an electric muffle The ash is mixed with 2 c c concentrated nitric acid and left overnight The excess of nitric acid is removed by evaporation and the residue redissolved in 10 c c 10 % hydrochloric acid The solution is made alkaline with strong ammonia, the precipitated iron hydroxide is centrifuged off, dissolved and precipitated again, and the fluid is transferred quantitatively into a separating funnel together with 5 c c 40 % solution of citric acid The fluid is made alkaline to litmus with ammonia and mixed with 10 c c 0 1 % aqueous solution of sodium diethyl dithio carbamate The yellow solution thus obtained is extracted with carbon tetrachloride and its copper content estimated as in the previous method

STATE OF COPPER IN RED BLOOD CORPUSCLES

The fact that copper cannot be removed from plasmolysed red blood corpuscles by dialysis in neutral solution was confirmed by analysis of the copper content of laked corpuscles before and after dialysis against distilled water The Cu content of the solution was 0 024 mg Cu per 10 c c corpuscles both before and after dialysis

This experiment clearly demonstrates that the copper of the red blood corpuscles is bound to an organic non-dialysable substance such as a protein Further work on purification of this compound supported this view and has led, moreover, to the isolation from red blood corpuscles of a copper-protein compound in a pure crystalline state This compound, which we have named *haemocuprein*[*] (Mann and Keilin 1938), contains the total copper present in erythrocytes

We propose now to describe the successive stages of purification of this compound

PURIFICATION OF HAEMOCUPREIN FROM RED BLOOD CORPUSCLES

(1) *Extraction with alcohol and chloroform*

Red blood corpuscles from 4 l of defibrinated ox blood are separated from serum, washed twice with 4 l isotonic saline solution and plasmolysed with an equal volume of distilled water

4 l of the solution thus obtained (fraction A) contains 720 g dry substance and 4 8 mg Cu, i e 0 00066 % Cu To 4 l of this solution are added 3 5 l cold 90 % alcohol and 250 c c chloroform, the mixture is shaken for 5 min , allowed to stand 10 min in ice and then filtered on a large Buchner funnel (30 cm diameter) through a filter paper covered with kieselguhr The residue is mixed with 1 l water and filtered in the same way

The joint filtrates consisting of 8 l of clear yellow, sometimes slightly pinkish solution (B) contain 34 g dry substance and 2 4 mg Cu, i e 0 007 % Cu

(2) *Precipitation with lead acetate*

8 l of cooled extract B is treated with saturated solution of lead acetate until no further precipitate is formed by the addition of a drop of this reagent to 2–3 c c of the filtrate Usually, 100–140 c c of saturated lead acetate is required for this purpose After 10 min standing, the precipitate is centrifuged off, suspended in about 200 c c of $M/3$ K_2HPO_4 and, after

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[*] The term haemocu*prein* should not be confused with haemocu*prin* of Schmitz (1931), which is a protein free copper derivative of haemocyanine

3 hr standing, centrifuged off The brown or reddish brown fluid which contains most of the haemocuprein is dialysed for 24 hr against distilled water, giving 500 c c of solution (C) free from phosphates

(3) *Purification with tricalcium phosphate*

500 c c of fluid C is mixed with 2 g of freshly prepared tricalcium phosphate gel, acidified to *p*H 6 6–6 7 and after 10 min standing centrifuged or filtered 550 c c of clear slightly greenish solution (D) thus obtained contains 3 4 g dry substance and 1 02 mg Cu, i e 0 03 % Cu

(4) *Precipitation with acetone*

Solution D is precipitated at *p*H 6 7–6 8 with 1 7–2 vol of cold acetone and one or two drops of 50 % $CaCl_2$ The precipitate is centrifuged off and dissolved in about 150 c c water The undissolved residue is removed by filtration leaving 150 c c of clear, distinctly greenish solution (E) containing 0 9 g dry substance and 0 58 mg Cu, i e 0 065 % Cu

(5) *Fractional adsorption on alumina Cγ*

Solution E is treated with two portions of alumina Cγ gel containing 50 mg dry substance per c c The first adsorption is carried out with only 10–15 c c of alumina gel which is centrifuged off giving a greenish yellow cake (F) The bluish solution is treated with the second portion of alumina gel, added in sufficient quantity to remove the whole colour of the solution The gel, which is centrifuged off, is of greenish blue colour (G)

Both coloured gels are eluted separately with 30 c c $0{\cdot}2M$ K_2HPO_4 each, alumina gels are centrifuged off and the coloured eluates (F and G) are dialysed against glass distilled water until completely free from phosphates, giving two coloured solutions (F and G) each 40 c c in volume

Solution F is opalescent and green, has 100 mg dry weight and contains 0 17 mg Cu, i e 0 17 % Cu

Solution G is clear and of bluish colour, has 160 mg dry weight and contains 0 38 mg Cu, i e 0 24 % Cu

(6) *Fractional precipitation by acetone*

Solution G only is used for further purification It is treated at *p*H 6 7 with one volume of acetone, the precipitate is centrifuged off while the fluid is treated with a second volume of acetone forming a second precipitate Both precipitates are separately dissolved in 5 c c distilled water, the undissolved residues are centrifuged off and rejected, while the fluids H and J are kept for the final stage of purification

(7) *Crystallization*

Of the two solutions thus obtained, the first (H) was found more suitable for crystallization of haemocuprein than the second (J), although in some preparations crystals could also be obtained from solution J

To 5 c c of clear blue solution H are added one or two drops of $M/1$ acetate buffer pH 6 and a sufficient amount of cold absolute alcohol to produce a slight opalescence The solution is left in the ice chest and the crystallization which begins almost at once is complete in 24–36 hr , the crystals settling down to form a distinctly blue sediment

The crystals thus obtained, washed with absolute alcohol and dried *in vacuo*, weigh 30 mg 4 4 mg of dried crystals contain 0 015 mg Cu, i e 0 34 % Cu

The analysis of crystals shows 14 35 % N, 1 12 % S and 0 34 % Cu The crystals are well defined but without sharp external faces, their general appearance is that of a double pyramid (fig 1, Plate, 11), on recrystallization they change their shape and become tabular with hexagonal outlines (figs 2, 3 and 4, Plate 11)

A preliminary determination of the sedimentation constant carried out at Oxford by Mr J St L Philpot shows that the protein of this preparation is homogeneous and that its molecular weight is not far from 35,000 It therefore appears to contain two atoms of copper per molecule

HAEMOCUPREIN FROM RED BLOOD CORPUSCLES OF OTHER MAMMALS

The method of preparation of haemocuprein from the red blood corpuscles of ox described above was applied to the corpuscles of other mammals In only two cases, however, were good preparations of this compound obtained, one from corpuscles of sheep, containing 0 22 % Cu, the other from corpuscles of horse and containing 0 31 % Cu Both preparations gave distinctly blue solutions

Assuming the copper content of pure copper protein in all animals to be 0 34 %, the purity of these two preparations would be 65 and 92 % respectively

The presence of a small amount of impurity prevented the complete crystallization from taking place On redissolving the precipitate and treating the solution with alcohol, an incomplete crystallization has been obtained giving a mixture of fine amorphous material, numerous crystals with rounded corners and only a few perfect crystals

The attempt to purify this compound from the red blood corpuscles of man* gave so far two preparations with the copper content of 0 187 and 0 21 %

HAEMOCUPREIN FROM BLOOD SERUM

The evidence that the copper in blood serum of mammals is present in a more or less loose combination with an organic non-dialysable substance such as a protein was already given in the first section of this paper

By applying to horse serum a method of purification slightly different from that already described, we have been able to obtain a serum copper-protein compound in a highly purified state The compound thus obtained was found to be also of a distinctly blue colour and having all the properties of haemocuprein obtained from corpuscles

We can therefore consider this compound if not identical, at least sufficiently similar to haemocuprein to be referred to under the same name

PURIFICATION OF HAEMOCUPREIN FROM HORSE SERUM

2 l horse serum is mixed with 2 l water, 3 6 l alcohol (90 %) and 400 c c chloroform The mixture is thoroughly mixed by whipping it for 5 min , left standing 10 min and then centrifuged 6400 c c of clear yellow fluid is collected and treated with 100 c c saturated solution of lead acetate The cake is eluted with alkaline phosphate, the mixture centrifuged, the fluid collected and dialysed against distilled water until free from phosphate 650 c c of clear greenish yellow solution thus obtained is mixed with 2 vol acetone and centrifuged The precipitate is dissolved in 200 c c water and treated for 30 min with a current of CO_2 The mixture is left overnight in the ice-chest, the precipitate centrifuged off and 200 c c of clear greenish solution collected which has 3 8 mg dry weight per c c contains 0 075 % Cu

The fluid mixed with the first lot of 15 c c alumina $C\gamma$, is centrifuged and the greenish grey cake rejected The clear fluid is now mixed with the second lot of 30 c c alumina $C\gamma$, centrifuged, the greenish blue cake collected, eluted with alkaline phosphate and dialysed 40 c c of fluid thus obtained has 6 mg dry weight per c c and contains 0 1 % Cu The adsorption on alumina $C\gamma$ is repeated once more, the phosphate eluate dialysed, and the fluid treated with one volume of acetone The precipitate is dissolved in water, the insoluble residue centrifuged off, the clear solution mixed with 2 vol of alcohol to give 30 mg of distinctly blue precipitate containing 0 25% Cu This preparation may therefore be considered as 75% pure On redissolving this precipitate in water and treating it with alcohol, a mixture of amorphous material and crystals is obtained

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* For the supply of human blood we are very grateful to Professor J Ryle and his staff

HEPATOCUPREIN THE COPPER-PROTEIN COMPOUND OF LIVER

It is well known that the largest concentration of copper in mammals is found in the liver, especially in the liver of young animals While the amount of copper per kg of fresh weight in the whole blood is from 2 to 4 5 mg, in skeletal muscle it is about 1 2 mg, in ox liver it reaches 21 5 mg, and in calf liver it may be as high as 44 1 mg (Lindow, Elvehjem and Peterson 1929)

It was shown by Gruzewska and Roussel (1937) that minced calf liver contains the same amount of copper before and after dialysis In other words, the copper present in liver is combined with an organic non-dialysable compound such as a protein By a fractional precipitation of liver extracts they obtained preparations containing 0 155% Cu (lamb liver) and 0 21% Cu (calf liver)

We have been able to confirm the main results obtained by these workers

By applying to liver a method of purification very similar to that which we have used for the red blood corpuscles of ox, we have been able to isolate from it a copper-protein compound containing 0 34% Cu Although this compound has the same copper content as the crystalline haemocuprein, it does not crystallize when submitted to the treatment which induces the crystallization of haemocuprein As it differs also from the latter in being almost colourless, we propose to refer to it at least temporarily under the name of *hepatocuprein*

ISOLATION OF HEPATOCUPREIN FROM OX LIVER

(1) *Extraction with water*

1750 g minced ox liver is mixed with 1750 c c water and left standing overnight in the ice-chest The mixture, after pressing out, gives 1650 c c of brownish red extract which contains 152 mg dry weight per c c and 0 0197% Cu

(2) *Extraction with alcohol and chloroform*

1600 c c of this extract is mixed with 1450 c.c of cold 90% alcohol and 160 c c chloroform, the mixture stirred 10 min and centrifuged 2500 c c of yellow opalescent fluid (B) thus obtained has 19 mg dry weight per c c and 0 031% Cu

(3) *Precipitation with lead acetate*

The cooled alcohol-chloroform extract is treated with saturated lead acetate until no further precipitate is obtained by this reagent The precipitate is centrifuged off, eluted with alkaline phosphate and dialysed, giving 800 c c of brownish solution (C) containing 1 46 mg dry weight per c c and 0 137 % Cu

(4) *Purification with tricalcium phosphate*

Solution C is mixed with 2 g tricalcium phosphate gel, adjusted to pH 6 7 and the gel filtered off, giving 800 c c of clear solution (D)

(5) *Precipitation with acetone*

Solution D is mixed with 1400 c c cold acetone, 1 c c $M/1$ acetate buffer pH 6 and 0 5 c c 50 % $CaCl_2$ After 10 min standing the precipitate is centrifuged off, dissolved in 170 c c water, neutralized, the insoluble residue rejected, leaving 170 c c of clear slightly greenish solution (E) containing 2 mg dry weight per c c and 0 24 % Cu

(6) *Fractional adsorption on alumina Cγ*

Solution E (160 c c), adjusted to pH 6 7, is treated with three successive lots of 10, 20 and 20 c c of alumina Cγ, the cakes of gel being centrifuged off after each treatment and eluted separately with alkaline phosphate The eluates, dialysed against distilled water, gave the following three solutions

Solution F 35 c c opalescent, slightly yellow, containing 5 5 mg dry weight per c c and 0 06 % Cu

Solution F_1 27 c c turbid fluid, containing 5 1 mg dry weight per c c and 0 16 % Cu

Solution G 23 c c clear slightly bluish fluid, containing 2 4 mg dry weight per c c and 0 25 % Cu

(7) *Precipitation with acetone*

20 c c of solution G is precipitated with 2 vol of acetone at pH 6 6, the precipitate dissolved in 10 c c glass distilled water, the undissolved residue centrifuged off, leaving a clear solution containing 0 34 % Cu

The copper content did not change after two successive reprecipitations with alcohol

1 mg of this hepatocuprein dissolved in 1 c c water shows hardly any colour, while the same concentration of haemocuprein is distinctly bluish

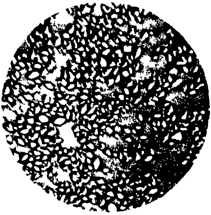

Fig. 1

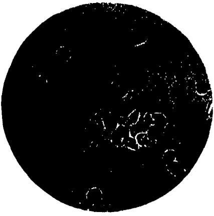

Fig. 2

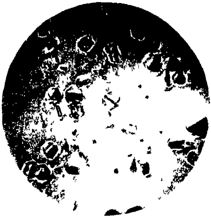

Fig. 3

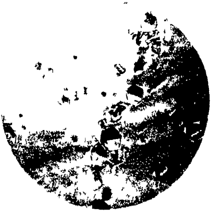

Fig. 4

Crystals of haemocuprein containing 0·34% Cu from red blood corpuscles of ox

Fig. 1. Material at first crystallization

Figs. 2–4. Material recrystallized second and third times

PROPERTIES OF HAEMOCUPREIN AND HEPATOCUPREIN

Having described the methods of isolation and purification of haemocuprein and of hepatocuprein we shall now summarize their general and distinctive properties The mere fact that very similar if not identical methods can be applied for the isolation of these compounds, independently of their origin, already indicates a great similarity in the properties of their protein Moreover, the solubility of these compounds and their behaviour towards different reagents, suggest that they belong to the class of albumins

The copper content of these compounds is 0 34 %, and this applies to the crystalline haemocuprein as well as to the highly purified hepatocuprein

The copper is, however, only loosely bound to the protein and can easily be separated from it by means of a mild reagent such as a dilute solution of trichloroacetic acid

For instance, 5 mg of haemocuprein containing 0 017 mg Cu, dissolved in 2 c c water gives a distinctly blue solution The addition of 0 5 c c 20 % trichloroacetic acid to this solution produces a white precipitate of protein which is centrifuged off and washed with dilute trichloroacetic acid This precipitate is found to be completely devoid of copper while the colourless protein-free supernatant fluid mixed with the washing of the precipitate contains 0 017 mg of inorganic copper

Fresh preparations of haemo- and hepatocuprein do not contain free inorganic copper In fact, the addition of diethyl-dithio-carbamate to such preparation does not reveal even a trace of copper Only in solutions kept for a long time or damaged in any way does the copper begin to appear in a free state

One of the distinctive properties of haemocuprein is its blue colour which is much stronger than that of an equivalent amount of a copper salt in alkaline solution In other words, the depth of its colour can hardly be accounted for by its copper content even if it would be present in a completely ionized state

The colour of haemocuprein disappears, however, when its protein is denatured and copper is liberated It also disappears almost completely when the compound is treated with $Na_2S_2O_4$ which reduces the copper This reduction appears to be irreversible as the colour does not reappear when the reduced solution is shaken with air, or is treated with a dilute solution of hydrogen peroxide

Hepatocuprein, which shares several properties in common with haemocuprein, differs however from the latter by being almost colourless. This dissimilarity may be due either to some differences in the structure of the proteins or to the difference in the valency of the copper We cannot dismiss, however, the possibility that in nature these two copper-protein compounds may be intimately connected, or even interconvertible

DISTRIBUTION OF COPPER-PROTEIN COMPOUNDS

It is conceivable that in all organisms the copper is present as a compound with an organic molecule, mainly the proteins In fact, there is no evidence that inorganic or free copper is ever present in measurable amounts in animals It is, however, difficult to determine what portion of the total copper-protein compound present in an organism can be assigned to haemo- or hepatocuprein That in blood the whole copper is present as haemocuprein seems to be supported by the facts that it is not dialysable, that it can be completely extracted with alcohol-chloroform mixture, and that it can easily be liberated from its compound with protein by a dilute solution of trichloroacetic acid (Tompsett 1934) If we accept for the copper content of blood the value of 2 mg /l , and as haemocuprein contains 0 34 % Cu, 1 l of blood must contain about 0 6 g of haemocuprein which corresponds to only 0 43 % of the haemoglobin present in the same volume of blood

It may be mentioned here that copper has also been found in measurable amounts in the crystals of haemoglobin (Elvehjem, Steenbock and Hart 1929), its concentration being between 0 019 and 0 034 mg per g of dry haemoglobin of horse Such a low value clearly shows that this copper does not form a part of the haemoglobin molecule (Elvehjem and others) It is therefore conceivable that it belongs to haemocuprein which was partly adsorbed by the crystals of haemoglobin

Whereas haemocuprein represents probably the total copper found in blood (red blood corpuscles and serum), hepatocuprein corresponds to only a portion of copper present in liver cells, the remaining copper being combined with some other proteins

Among other tissues of animals we have tested only heart muscle and found a small amount of a copper-protein compound which can be extracted with alcohol-chloroform mixture The purification of this compound, which was not carried very far, gave however, a fraction containing 0 06 % Cu As in the case of haemocuprein, the copper is easily liberated from this compound by treating it with a dilute solution of trichloroacetic acid These few considerations suggest that we are dealing here with a compound very similar to haemocuprein

That copper is very widely if not universally distributed in plants was demonstrated by several workers To Lindow, Elvehjem and Peterson (1929) we owe the numerical data of copper estimations in more than 100 different plants used as food On the other hand, it was shown recently that at least one enzyme, widely distributed in plants, the polyphenol oxidase, is a copper-protein compound (Kubowitz 1937, Keilin and Mann 1938) The study of this oxidase in mushrooms has revealed, however, that the copper of the oxidase represents hardly 2% of the non-dialysable copper present in mushrooms (Keilin and Mann 1938) It is therefore possible that at least a fraction of the remaining copper of plants may exist as a copper-protein compound similar to haemocuprein or hepatocuprein

BIOLOGICAL SIGNIFICANCE OF HAEMO- AND HEPATOCUPREIN

The few tests to which we have submitted these compounds in order to detect some possible biological reactions or catalytic properties have so far given only negative results

We have found for instance, that the purified preparations of haemo- and hepatocuprein do not combine loosely with oxygen as does haemocyanine They do not promote phosphorylation reactions and do not catalyse directly any one of the reactions catalysed by polyphenol oxidase, cytochrome oxidase, peroxidase, catalase or carbonic anhydrase

They may still catalyse a reaction which we have not yet tested, or may require for their catalytic activity some components which have been destroyed or left behind during the process of purification of these compounds

Although these compounds do not exhibit any one of the above mentioned catalytic properties, they must nevertheless have some definite physiological significance This is strongly supported by numerous investigations which have revealed the importance of copper in the life of different organisms, apart from its role as a constituent of haemocyanine and of polyphenol oxidase

By properly controlled feeding experiments of higher organisms and cultivation of micro-organisms the importance of copper was well demonstrated in the formation of haemoglobin, the growth of micro-organisms, plants and higher animals and the development of cytochrome, cytochrome oxidase and chlorophyll *

It is certain that some of the copper supplied to organisms with their food is utilized for building up haemo- or hepatocuprein The formation of

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* For the rich literature bearing on this subject the reader is referred to an excellent review by Elvehjem (1935)

these compounds may therefore represent one of the steps in copper metabolism and may directly or indirectly be responsible for some of the physiological effects which are rightly ascribed to copper

Haemo- and hepatocuprein can be added now as two new biological organic copper compounds to the list of three other compounds previously known Of these three compounds, haemocyanine and polyphenol oxidase are copper-protein compounds, while turacine is a copper uroporphyrin compound

SUMMARY

1 *Haemocuprein* is a blue copper-protein compound present in the red blood corpuscles and serum of mammals

2 A method for isolation of this compound in a pure crystalline state from the red blood corpuscles of ox is described

3 A highly purified and partly crystalline haemocuprein may also be obtained from the red blood corpuscles of sheep and horse and the blood-serum of horse

4 Pure haemocuprein contains 14 35 % N, 1 12 % S and 0 34 % Cu

5 An almost colourless copper-protein compound, *hepatocuprein*, containing 0 34 % Cu can be isolated from ox liver

6 Haemo- and hepatocuprein have several properties in common Both have the same copper content, can be extracted from the crude material with the same alcohol-chloroform mixture and purified by the same method In both compounds the copper is only loosely combined with the protein and is easily liberated by dilute trichloroacetic acid

7 They do not combine with molecular oxygen and do not exhibit any of the catalytic properties of enzymes such as peroxidase, catalase, polyphenol and cytochrome oxidase or carbonic anhydrase

8 The possible biological significance of haemo- and hepatocuprein is discussed in relation to the known effects of copper on blood formation, growth and metabolism of organisms

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A comparative study of the development *in vivo* and *in vitro* of rat and rabbit molars

BY S GLASSTONE

Strangeways Research Laboratory, Cambridge

(*Communicated by Sir Edward Mellanby, F R S —Received* 30 *March* 1938)

[Plates 12–14]

INTRODUCTION

Although the normal embryology of mammalian teeth has been carefully studied, little is known of the developmental mechanics of teeth

The present communication is concerned with the problem of cusp formation The main object of the investigation was to find how far the formation of molar cusps was due to extrinsic factors in the jaw and how far to intrinsic factors in the tooth germ itself Previous work (Glasstone

1936) had shown that embryonic teeth grown *in vitro* and removed from the general influence of the body continue to develop In these earlier experiments the rudiments were explanted when cusps had already appeared but before odontoblasts and dentine had differentiated In the present experiments the tooth germs were explanted at an earlier stage before the cusps had begun to form, to see whether cusps would develop *in vitro* in the isolated rudiment and if so whether they would correspond in number, shape and arrangement with those of the normal embryonic tooth

The most convenient material for these experiments was the first lower permanent molar of embryonic rats and the two lower temporary molars in embryonic rabbits To provide a standard of comparison for the experimental results, it was necessary to make a detailed histological and anatomical study of the normal embryology of these teeth in the rat and rabbit, since there is no complete account of the embryonic development of the teeth in these two forms

The first part of this communication therefore deals with the normal development of the first lower molar in the rat and the two lower temporary molars in the rabbit from the first appearance of the dental lamina to the end of cusp formation The development *in vitro* of these molars from both animals is then described and cusp formation in the normal jaw and in the explanted rudiments are compared

Although there is a fairly large literature on the morphology of the post-embryonic teeth in rodents, there are comparatively few papers on the embryonic development of the teeth in this group

Mahn (1890) describes the later stages of molar development in the mouse and is mainly concerned with the evolution of teeth of persistent growth

Tims (1901) gives a detailed account of the anatomical development of teeth of the embryonic guinea-pig and showed that molar (cheek) teeth of the Rodentia are multituberculate in origin and not trituberculate

Friant (1933) describes very briefly the gross anatomy of the temporary molars in the new-born rabbit

Harman and Smith (1936) give a detailed histological description of tooth development in the guinea-pig, with the object of studying the effects of diet on these teeth

PART I NORMAL DEVELOPMENT

Material and methods

Forty rats ranging in age from the 13th day of gestation to the 2nd day after birth and thirty-nine rabbits from the 15th to the 26th day of foetal life were used. The degree of development varies in different litters of the same age and in members of the same litter, so that in the following description the days given for various stages of development are approximate only.

The entire jaw was fixed in 3% acetic Zenker's solution, washed, dehydrated, cleared in methyl benzoate and embedded in paraffin or celloidin. The jaw of the 26-day foetal rabbit was decalcified in 5% nitric acid after fixation. Serial sections were cut and were stained with iron haematoxylin and chromotrop, with the exception of one series of the jaw of a 2-day rat which was stained with azan.

Wax reconstructions were made of five dentine papillae of rats from the 17th day of foetal life to the 2nd day after birth and of five dentine papillae of rabbit embryos from the 20th to the 26th day of foetal life. The reconstructions were made as follows:

Serial sections of the tooth germs were cut at a thickness of 6μ and a projection drawing of such sections was made on unwaxed blotting paper. The blotting paper was 483μ in thickness when waxed so the sections were projected at a magnification $\frac{483}{6} = 80.5$ diameters. After the outlines had been drawn, the areas of dentine were coloured red with safranin and those of the enamel were stained with light green. The blotting paper was then soaked in molten paraffin wax, the outlines were cut out and the plates were stuck together in serial order.

The development of the first permanent lower molar of the rat

The following terms are used in describing the orientation of the teeth: anterior, proximal, posterior, distal, towards the cheek, buccal, towards the tongue, lingual.

The first signs of tooth development appear at about the 13th day of foetal life as a local thickening in the epithelium of the mouth; this thickening forms a narrow continuous band in the molar region only (fig. 10, Plate 12).

About 24 hours later the thickened epithelium has penetrated into the underlying tissue to form the general dental lamina. In transverse (coronal) section it can be seen that this lamina consists of two layers of cubical epithelium including between them a dense mass of irregularly arranged flattened epithelial cells At the buccal side of the inner growing end of the lamina there is a bud-shaped thickening which represents the first appearance of the tooth germ (fig 11, Plate 12)

By the 15th day the small, bud-shaped tooth germ of the previous stage has enlarged very greatly in relation to the rest of the lamina It has become wider and deeper and is now shaped something like an inverted saucer (fig 12, Plate 12) The two layers of cubical cells are much more distinct, the superficial layer now being recognizable as the external enamel epithelium and the lower layer as the internal enamel epithelium The cells between the two layers, which constitute the future enamel pulp, are much compressed The concavity of the internal enamel epithelium is filled with a mesodermal condensation which represents the early dentine papilla

As the tooth enlarges, the space between the internal and external enamel epithelia, containing the flattened epithelial cells, widens (fig 13, Plate 12)

At the margin of the future enamel pulp, these flattened cells are being replaced by a loose network of cells and fibres, the stellate reticulum or enamel pulp A layer of flattened cells, the stratum intermedium, separates the enamel pulp from the external enamel epithelium The two enamel epithelia are connected in the centre of the tooth by a cone-shaped condensation of flattened epithelial cells, the enamel cord

By the 17th day of gestation, the lower molar appears as an elliptical body which as yet shows no trace of cusps and is still connected with the dental lamina (fig 1) The flattened cells of the enamel pulp have now been replaced by stellate reticulum, except in the region of the enamel cord

Fig 1 Drawing of a wax reconstruction of the dentine papilla of a molar tooth germ from a 17 day rat foetus Cusps have not yet appeared

Cusps begin to appear at about the 18th day as a slight dipping of the internal enamel epithelium into the dentine papilla Forth-eight hours later, two cusps, the disto-lingual and middle lingual have become distinct (fig 2) The tooth is extending in both the buccal and proximal (anterior) directions and the first signs of three more cusps are distinguishable, viz the disto-buccal and middle buccal, which are parallel with the disto-lingual and middle lingual respectively, and the proximo-lingual cusp, which is slightly more advanced than the buccal cusps The middle lingual cusp almost touches the external enamel epithelium, elsewhere the enamel pulp has enlarged and the enamel cord has disappeared As the three lingual cusps grow deeper they begin to slope in a proximal direction At about the 21st day, a sixth cusp, the third buccal, begins to form adjacent to the proximo-lingual cusp Blood vessels now penetrate the external enamel epithelium, the cells of which are losing their cuboidal shape and becoming flattened and enter the stellate reticulum At the tips of some of the cusps the odontoblasts are beginning to differentiate beneath the internal enamel epithelium

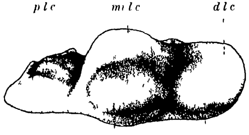

FIG 2 Drawing of a wax reconstruction of the dentine papilla of a molar tooth germ from a 20 day rat foetus (buccal view) Note the presence of the disto lingual and middle lingual cusps, and the first signs of the proximo lingual, the disto buccal and the middle buccal cusps *d b c* disto buccal cusp, *d l c* disto lingual cusp, *mi b c* middle buccal cusp, *mi l c* middle lingual cusp, *p l c* proximo lingual cusp

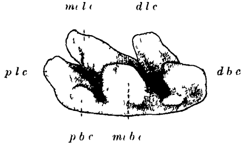

FIG 3 Drawing of a wax reconstruction of the dentine papilla of a molar tooth germ of a newly born rat (buccal view) The three lingual cusps are now well developed and the three parallel buccal cusps are present *d b c* disto buccal cusp, *d l c* disto lingual cusp, *mi b c* middle buccal cusp, *mi l c* middle lingual cusp, *p b c* proximo buccal cusp, *p l c* proximo lingual cusp

At birth six cusps are present three large lingual and three small buccal cusps (fig 3) The fissures between the cusps are much deeper in the linguo-buccal than in the proximo-distal direction and so divides the cusps into three groups, each group having a lingual and buccal cusp The apices of all the lingual cusps are in contact with the external enamel epithelium which is beginning to degenerate Blood vessels are penetrating deeper into the stellate reticulum The cells of the internal enamel epithelium have begun to differentiate into true ameloblasts They have become more columnar and their nuclei, which lie in the outer parts of the cells, are more oval

Twenty-four hours after birth, the buccal cusps have grown nearly as large as the lingual Two, very small additional cusps have appeared, one immediately behind the two distal cusps and the other buccal to the disto-buccal cusp The external enamel epithelium has now completely degenerated The ameloblasts are still more columnar and the first traces of enamel formation are visible A continuous layer of dentine covers most of the tooth

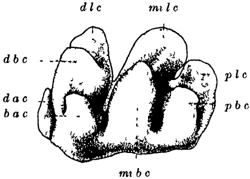

Fig 4 Drawing of a wax reconstruction of the dentine papilla of a molar tooth germ of a 2 day old rat (buccal view) The buccal cusps have grown nearly as large as the lingual cusps and they are all now cone shaped Note the presence of the two additional cusps, one immediately behind the two distal cusps and the other buccal to the disto buccal cusp *b a c* buccal additional cusp, *d a c* distal additional cusp, *d b c* disto buccal cusp, *d l c* disto lingual cusp, *mi b c* middle buccal cusp, *mi l c* middle lingual cusp, *p b c* proximo buccal cusp, *p l c* proximo lingual cusp

By the 2nd day after birth, the tooth has attained its final shape The cone-shaped cusps are covered by a considerable layer of dentine and in some places by a layer of enamel (fig 4)

The development of the temporary molars in the rabbit

In the foetal rabbit the first thickening of the mouth epithelium appears at about the 15th day The subsequent early development is essentially the same as in the rat The dental lamina in the molar region has penetrated into the mandible and the tooth germs are formed at the inner growing end in the manner described above

By the 17th day the enamel organ has become bell-shaped and the underlying medoserm has begun to form the dentine papilla Twenty-four hours later the tooth is spherical and the stellate reticulum is differentiating at the margins of the enamel pulp and a conical enamel cord connects the two layers of the enamel epithelium in the middle of the tooth

In the 20-day foetus the stellate reticulum has differentiated almost throughout the enamel pulp and the enamel cord is being replaced by stellate cells The cells of the external enamel epithelium are beginning to flatten whilst those of the internal enamel epithelium are still cubical (fig 5 and fig 15, Plate 13)

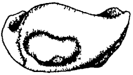

FIG 5 Drawing of a wax reconstruction of the dentine papilla of a molar tooth germ from a 20 day foetal rabbit (buccal view) The cusps have not yet appeared

The cusps begin to appear at about the 21st day In the distal third of the tooth the enamel epithelium dips downwards dividing the superficial part of the tooth into a distal mass, the rudiment of the single distal cusp, and a larger, proximal mass which is the rudiment of the proximal group of cusps

Growth proceeds actively in the proximo distal and bucco lingual directions and less vigorously in a vertical direction The distal cusp slopes backwards slightly, by the 22nd day it is separated from the proximal group by two oblique fissures, one from the buccal and one from the lingual side, which meet in the mid-line (fig 6) There is a shallow depression in the middle of the distal cusp suggesting a subdivision into two smaller cusps The proximal group consists of three parts there is a large central portion, in which the enamel epithelium dips down as if about to form three lesser cusps, on the buccal side of this mass there is a single cusp and on the lingual side are a pair of cusps The enamel cord has disappeared and odontoblasts are present in the tips of the cusps Dentine begins to form about 24 hours later

The cusps continue to elongate and in a 24-day foetus are in contact with the external enamel epithelium, also the ameloblasts are more columnar

By the 26th day, anatomical development is almost completed (fig 7) The tooth has enlarged considerably but most of the growth has taken place in a vertical direction The two oblique distal fissures now form a single, straight fissure running in a bucco-lingual direction The distal cusp is no longer oblique but has become vertical, and at the same time has

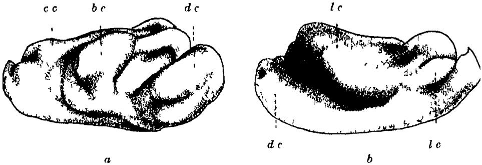

Fig 6 *a* Drawing of a wax reconstruction of the dentine papilla of a molar tooth germ from a 22 day rabbit foetus (buccal view) showing the large single distal cusp, the central cusp of the proximal group and the single buccal cusp

b Same as *a* showing the lingual side Note the two lingual cusps of the large proximal group *b c* buccal cusp, *c c* central cusp, *d c* distal cusp, *l c* lingual cusp

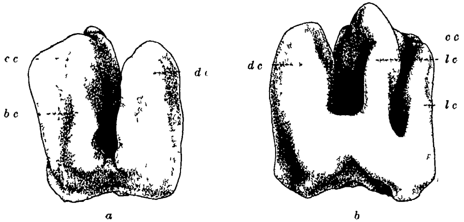

Fig 7 *a* Drawing of a wax reconstruction of the dentine papilla of a molar tooth germ of a 26 day rabbit foetus (buccal view) The cusps are now more vertical and the distal fissure is much deeper

b Same as *a*, showing the lingual side *b c* buccal cusp, *c c* central cusp, *d c* distal cusp, *l c* lingual cusp

lost the faint traces of two secondary cusps seen at an earlier stage The proximal group of cusps is now vertical and in the central cusp the slight indication of three secondary cusps has disappeared The external enamel epithelium has begun to degenerate and connexion with the dental lamina has been lost The stellate reticulum is also degenerating, necrosis beginning at the outer surface and spreading inwards Numerous blood vessels occur near the degenerate area The first temporary molar is a little smaller than the second and the number and general arrangement of the cusps is essentially the same in both, but in the first molar the proximal group of cusps is a little more elongated in a proximo-distal direction

A comparison of molar development in the rat and in the rabbit

Although a description is given of teeth of different series, that is the permanent molar of the rat and the temporary molar of the rabbit, a comparison of their early development is of interest as there are some slight histological differences

In the rat the tooth germ is elliptical, but in the rabbit it has the usual bell-shape The enamel cord is better defined in the rat and persists for a day after cusp-formation has begun, whilst in the rabbit it degenerates when the cusps first appear In the rat the cells of the external enamel epithelium remain cuboidal until they begin to degenerate, but in the rabbit they lose their cubical form before the cusps appear According to Addison and Appleton (1922) blood vessels penetrate the enamel organ of the rat at about the 21st day of gestation, two days before enamel develops In the rabbit, on the other hand the present writer has found that blood vessels do not penetrate the enamel organ at any stage in the development of the tooth, although numerous blood vessels are present near the degenerating stellate reticulum at the time of enamel formation

PART II DEVELOPMENT *IN VITRO*

Material and methods

The explants were all obtained from foetuses, as it was found impossible to remove developing teeth aseptically from animals after birth

The first lower permanent molar of the rat and the first and second temporary lower molars of the rabbit were cultivated Since cusp formation in these teeth begins at the 18th day of foetal life in the rat and at the 21st day in the rabbit, 17-day rat embryos and 20-day rabbit embryos were used It is more difficult to remove the tooth germs from the foetal jaw in the rat than in the rabbit, as the rat molar rudiment is smaller and less distinct than that of the rabbit and less readily freed from the mouth epithelium and surrounding tissue Consequently some of the rat molars were greatly damaged by the dissection and degenerated after a few days *in vitro* whilst others developed rather abnormally

To determine the stage of development of each rudiment when first explanted, one tooth was removed for cultivation, and the corresponding tooth from the opposite ramus was fixed either *in situ* or after dissection, and sectioned to serve as a control

The tooth germs were grown by the hanging drop method on 1¼ in square cover-slips and were explanted with either the buccal or the lingual surface against the glass The culture medium consisted of three drops of fowl plasma and one drop of extract of an 11-day fowl embryo the extract was made with Pannett and Compton's saline containing 1 % glucose The cultures were incubated at 37° It was found that if the explants were transferred to fresh medium every 72 hours they contracted considerably and much better results were obtained by leaving them undisturbed for 4–6 days

Sixty-nine tooth germs were fixed after intervals of 1–9 days' cultivation and examined histologically They were removed from the clot and immersed in 3 % acetic Zenker's solution for 30 min , then washed, dehydrated, cleared in methyl benzoate and embedded in paraffin wax The controls were similarly treated All sections were stained with iron-haematoxylin and chromotrop

Wax reconstructions of 14 explants were made after 4, 6 and 9 days' cultivation

The development in vitro *of the first lower molar in the rat*

The explants consisted of the small, elliptical tooth germ which had been removed from the dental lamina (fig 1)

The tooth germ underwent a surprisingly normal histological and anatomical development during cultivation *in vitro* During the first 2 days in culture, the tissue was too opaque for detailed observation to be made on the living material, but after 24 hours it was just possible to distinguish the internal enamel epithelium growing downwards about half-way along the dentine papilla to form the first two cusps, viz the middle and disto lingual By the end of the 2nd day these cusps were quite well developed In sections many mitotic figures were seen in both the external and internal enamel epithelium they were more numerous in the internal enamel epithelium which was growing rapidly to form the cusps There was no indication that the cells of the enamel organ or the dentine papilla were dividing more actively in any particular region of the tooth, e g in the tips of the cusps

By the 4th day the rudiments had enlarged horizontally in what was anatomically the proximal direction, to form the third or proximo-lingual cusp If the enamel organ had been damaged by dissection, development was sometimes retarded so that only two cusps had appeared at this stage or the surface of the cusps were slightly convoluted in an abnormal way

During the next 24 hours the fissures between the lingual cusps quickly deepened, owing to the simultaneous downward growth of the internal enamel epithelium and upward growth of the dentine papilla At the same time the basal region grew out to form the first rudiment of the buccal cusps

After 6 days' growth the fissures between the lingual cusps were so deep that they almost cut the tooth into three The proximal, middle and distal buccal cusps had now developed in line with the three corresponding lingual cusps thus forming three parallel sets of cusps (fig 8) Two smaller cusps had also appeared, one behind the distal cusps, and the other between the middle and distal buccal cusps, but nearer to the median line than in the normal As in the normal, all the cusps sloped in a proximal direction At this stage mitosis was more abundant in the tips of the cusps than in the base of the tooth owing to the fact that the cusps were growing in all three directions, while the basal part of the tooth was only growing in length and width but not in height Odontoblasts were beginning to form

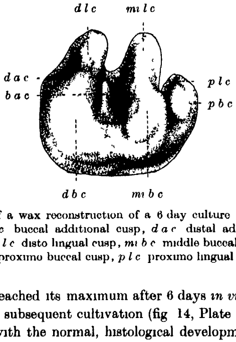

FIG 8 Drawing of a wax reconstruction of a 6 day culture of a rat tooth germ (buccal view) *b a c* buccal additional cusp, *d a c* distal additional cusp, *d b c* disto buccal cusp, *d l c* disto lingual cusp, *mi b c* middle buccal cusp, *mi l c* middle lingual cusp, *p b c* proximo buccal cusp, *p l c* proximo lingual cusp

Development reached its maximum after 6 days *in vitro* and proceeded no further during subsequent cultivation (fig 14, Plate 13)

As compared with the normal, histological development was considerably retarded *in vitro* Thus, after 6 days' cultivation, odontoblasts had only just begun to form, whereas in the normal tooth of equivalent age, i e 2 days after birth, odontoblasts, dentine and enamel were present

A comparison of the gross anatomical structure of the normal and explanted molars showed a remarkable correspondence The number, shape and arrangement of the cusps were almost identical in both and development *in vitro* was only retarded by about 24 hours so that a 6-day culture was comparable with the tooth of a day-old rat

During cultivation the teeth enlarged to about three times their original size This final size was less than half that of the normal tooth of the same age

Development in vitro *of the temporary molars of the rabbit*

As in the rat cultures the original explant consisted of the oval tooth germs isolated from the dental lamina (fig 5 and fig 15, Plate 13) During the 1st day's cultivation, the epithelium in the distal third of the tooth penetrated into the dentine papilla and formed a fissure dividing the tooth into a proximal part comprising about two-thirds of the tooth, and a distal part

By the 3rd day the rudiment had extended laterally and the original distal fissure formed two oblique prolongations, one running in a proximo-lingual and the other in a proximo-buccal direction The distal cups which sloped towards the distal end of the tooth displayed a slight central depression as if two secondary cusps were about to form In the large, proximal part several cusps had begun to develop a central cusp, two very early lingual cusps and a single buccal cusp Odontoblasts were differentiating

In the 4-day cultures the proximal group of cusps varied somewhat in different explants In some the pattern of the cusps was of the normal type as in the 3-day cultures, but in others an extra cusp had appeared proximal to the central one By this stage the cells of the internal enamel epithelium had begun to differentiate into functional ameloblasts A thin layer of dentine had now been deposited at the tip of the central cusp

The tooth germs reached their maximum development by the 6th day The fissures between the cusps were much deeper and more upright and the distal fissure had lost its previous obliquity and now ran straight across the tooth (fig 16, Plate 13) Sometimes the central cusp had become subdivided into two or three smaller cusps (fig 9*b* and fig 17, Plate 14) The two lingual and one buccal cusps were much better developed and in cultures containing the extra, proximal cusp the fissure between this and the central cusps was much deeper (fig 17, Plate 14) A thin layer of dentine had been deposited on the tips of all the cusps and some of the

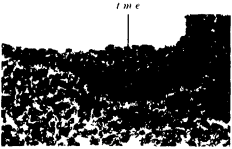

Fig 10

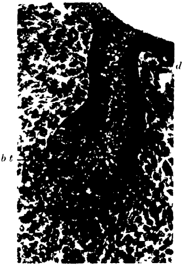

Fig 11

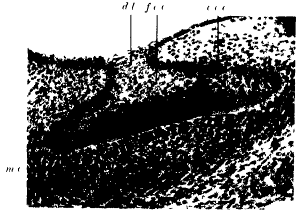

Fig 12

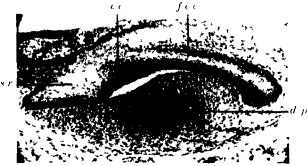

Fig 13

Fig 10 Transverse (coronal) section through the jaw of a 13 day rat foetus showing the thickening of the mouth epithelium (× 300)

Fig 11 Transverse (coronal) section through the jaw of a 14 day rat foetus showing the dental lamina and the bud shaped thickening at its inner end which represents the first appearance of the tooth germ (× 320)

Fig 12 Longitudinal section through the lower jaw of a 15 day rat foetus showing the enlarged enamel organ now shaped like an inverted saucer and condensation of the mesodermal tissue to form the dentine papilla (× 232)

Fig 13 Longitudinal section through a molar tooth germ from a 16 day rat foetus showing the enamel cord and the replacement of the flattened cells by the stellate reticulum (× 120)

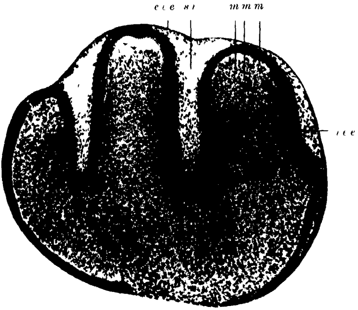

FIG. 14.

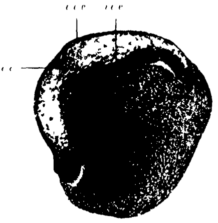

FIG. 15.

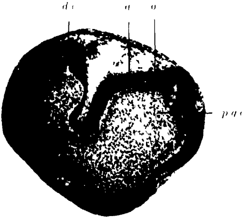

FIG. 16.

FIG. 14. Longitudinal section of a 6 day culture of a rat molar tooth germ. Note the numerous mitoses in the rapidly growing cusps. The cells of external enamel epithelium have lost their cuboidal shape. (× 140.)

FIG. 15. Longitudinal section of a normal control molar tooth germ of a 20 day rabbit foetus. The cusps have not yet appeared: there are no odontoblasts and the cells of the internal enamel epithelium have not yet differentiated into functional ameloblasts. (× 125.)

FIG. 16. Longitudinal section of a 6 day culture of a rabbit molar tooth germ. This culture formed the normal number of cusps: the single distal and the larger proximal group. Note the presence of odontoblasts and ameloblasts. (× 120.)

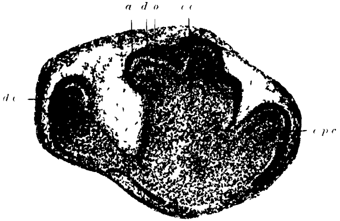

Fig 17

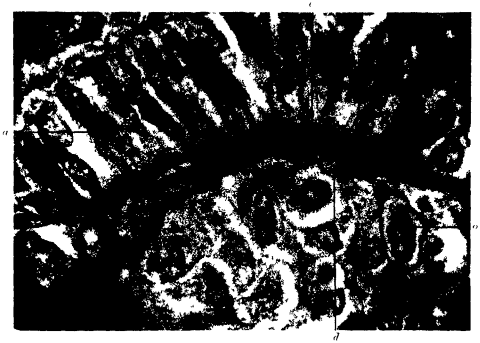

Fig 18

Fig 17 Longitudinal section of a 8 day culture of a rabbit tooth germ. In this culture the single distal and in the proximal portion an extra proximal cusp have formed. Also the central cusp has divided into three. Odontoblasts dentine and ameloblasts have formed during cultivation (× 84)

Fig 18 Longitudinal section of an 8 day culture of a rabbit molar tooth germ. During cultivation odontoblasts have differentiated and a thin layer of dentine has been deposited. Functional ameloblasts were present and they have formed enamel (× 1400)

odontoblasts were degenerate Most of the dentine had normal tubular structure but some was amorphous In places the ameloblasts had formed enamel in which the characteristic prisms could be distinguished (fig 18, Plate 14)

As in explants of the rat molars mitosis occurred throughout the culture period but in the early stages of growth it was not more abundant in any particular region In the latter part of the culture period when growth was largely restricted to the cusps mitosis became more active in this part of the tooth than towards the base

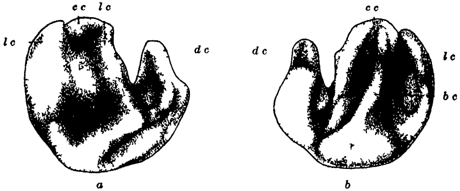

FIG 9 *a* Drawing of a wax reconstruction of the dentine papilla of a 6 day culture (lingual view) Note the single distal cusp and the two lingual cusps formed During cultivation this culture formed a considerable amount of dentine

b Same as above showing the buccal side In this culture the central cusp has subdivided *b c* buccal cusp *c c* central cusp *d c* distal cusp *l c* lingual cusp

When the anatomical structure of the explanted and the normal rabbit molar are compared the general distribution and shape of the cusps are found to be very similar In both the tooth is subdivided by a deep fissure into a smaller distal and a larger mesial portion and in both the proximal part consists of a central group of cusps two lingual cusps and a buccal cusp The explant differs from the normal in two chief respects (*a*) in the common occurrence of an additional cusp (fig 17 Plate 14) and (*b*) in a frequent subdivision of the central cusp into two or three secondary cusps (fig 9*b* and fig 17 Plate 14) Most of the cultures formed extra cusps but a few were quite normal (fig 16 Plate 13)

DISCUSSION

One of the most interesting and obscure problems in the development of molars is the mechanism of cusp formation

The tissue culture experiments show that the development of the cusps is a self-differentiating process Since the cusps which appear *in vitro* are almost normal in shape, number and arrangement, it is clear that the factors responsible for their development are intrinsic in the tooth germs and entirely independent of external mechanical conditions in the jaw

The nature of these intrinsic factors is not known It was thought at first that localized areas of increased mitotic activity in the enamel organ or in the dentine papilla might account for the enamel epithelium invaginating in certain definite regions to form the fissures No such areas of unusually active cell division could be found, however, in the normal or explanted rudiments As described above, when the cusps have already appeared and are growing rapidly mitosis is more abundant in the cusp region than in the base of the tooth, but the dividing cells are distributed throughout the cusps and are not more numerous in any special areas

A curious feature of the rabbit explants is the formation of additional cusps during cultivation (fig 9*b* and fig 17, Plate 14) It is noteworthy that the distribution of these extra cusps is remarkably constant in different cultures and that they only occur in the large central cusp of the proximal group In the normal 22-day foetus, as previously stated, this central cusp shows faint traces of secondary cusps which later disappear It is probable, therefore, that the extra cusps which appear *in vitro* are only an exaggeration of the poorly developed transitory cusps which occur in the normal tooth germ It is possible that this exaggeration may be due to the fact that the tooth germ is embedded in a semi-solid medium which would tend to inhibit its lateral expansion As the rudiment is explanted with either its buccal or its lingual surface against the glass, enlargement would be most restricted along the bucco lingual axis, which would tend to cause the expanding epithelium to grow inwards in this direction It would not be very surprising if this ingrowth should occur where small indentations of the enamel epithelium already existed If the ingrowth bears no relation to pre-existing structures, it is difficult to understand why the form and arrangement of the extra cusps should show so little variation in different cultures

In the rat explants, extra cusps do not develop during cultivation This may be due to the fact that the fissures between the cusps are normally very deep so that the epithelium is able to expand without disturbing the normal morphology of the tooth

Although anatomical development was more normal in the explanted molars of the rat than in those of the rabbit, histological development was more normal in the rabbit explants Thus in the rabbit molars the formation of odontoblasts and dentine proceeded at almost the normal rate whilst in the rat molars both these processes were greatly retarded, enamel also differentiated in the rabbit but not in the rat explants Why the rat and rabbit molars should behave differently *in vitro* is not clear as precisely the same culture methods were used for both

The author wishes to express her thanks to Dr H B Fell for her continued help shown throughout the work, and for the drawings of the wax reconstructions Also to the Medical Research Council by whom the expenses of the investigations were defrayed, and to the Ella Sachs Plotz Foundation

This work was carried out whilst receiving a personal grant from the Medical Research Council

SUMMARY

1 The normal development of the first lower molar of the rat has been described from the 13th day of foetal life to the 2nd day after birth

2 The normal development of the lower temporary molars of the rabbit has been described from the 15th to the 26th day of foetal life

3 Molar tooth germs of the rat and rabbit when cultivated *in vitro* showed remarkable powers of anatomical and histological development

4 The tooth germs were explanted before cusps had appeared and during cultivation formed cusps which were almost normal in shape, number, and arrangement

5 Odontoblasts and dentine differentiated in the cultures

6 In the explants of rabbit tooth germs the internal enamel epithelium differentiated into ameloblasts which formed enamel

ABBREVIATIONS USED ON PLATES 12–14

a	ameloblasts	*e p c*	extra proximal cusp
b t	bud shaped thickening	*f e c*	flattened epithelial cells
c c	central cusp	*i e e*	internal enamel epithelium
d	dentine	*m*	mitosis
d c	distal cusp	*m c*	mesodermal condensation
d l	dental lamina	*o*	odontoblasts
d p	dentine papilla	*p g c*	proximal group of cusps
e	enamel	*s r*	stellate reticulum
e c	enamel cord	*t m e*	thickened mouth epithelium
e e e	external enamel epithelium		

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An analysis of the mechanism of oestrogenic activity

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(*Communicated by F H A Marshall, F R S* – *Received* 8 *June* 1938)

INTRODUCTION

The series of brilliant investigations involving the chemical identification of naturally occurring oestrogens is now biochemical history But our knowledge of the mode of action of these compounds is fragmentary, we know pretty well what they do, but not how they do it The discovery that certain synthetic compounds differing definitely in structure and chemical properties from the native hormones (Cook, Dodds, Hewitt and Lawson 1934, 1936, Dodds 1936, Pincus and Werthessen 1936, Robson and Schonberg 1937, and others) seemed to afford an opportunity to obtain an insight into the mechanism of oestrogenesis We were extremely fortunate in securing the co operation of Dr Louis F Fieser and his co-workers who generously made available to us a series of synthetic compounds having presumable oestrogenic potency We have employed these substances in a set of tests in which the native compounds show characteristic activity, and, on the basis of the derived data, have attempted to set up some unifying hypothesis to account for the varied types of behaviour of these compounds and the hormones *in vivo*

METHODS OF ASSAY

The conventional Allen-Doisy technique for the determination of the comparative oestrogenic activity of various materials has certain disadvantages Since it requires the subcutaneous injection of the oestrogens, the rate at which these compounds are absorbed may become a complicating factor Thus Deansley and Parkes (1933) found that oestrin in oily solution was retained for a considerable length of time at the site of subcutaneous injection More recently (Deansley and Parkes 1937) they report that the marked difference in potency between oestrone and oestrone benzoate disappears upon intravenous injection, and on the basis of this and similar observations made with testosterone and certain of its esters they conclude that the solubility of the hormones in body fluids is the prime determinant of their potency In studying the oestrogenic activity of various synthetic compounds Cook, Dodds, Hewitt and Lawson (1934) were forced to modify the regular Allen-Doisy technique in order to obtain evidences of the activity of certain of their compounds Finally, to make significant quantitative comparisons between various oestrogens large numbers of animals are required (cf Coward and Burn 1927, and others), and when adequate data are accumulated we know merely that, judging by the nature of the vaginal smear at a given number of hours after the first injection, compound A is more or less active than compound B This in effect disregards possibilities of difference due to differences in the time course of their reactions which potent substances may very well exhibit

METHOD OF ASSAY WITH OVARIECTOMIZED MICE

Since intravenous injection into mice is a laborious and time-consuming affair we have resorted to intraperitoneal injection to insure maximum hormone absorption Our technique with mice is as follows

The material to be injected is dissolved in olive oil in such concentration that the dose per mouse is contained in 0 4 c c of solution The test animals are injected in groups of five either at 14 days after ovariectomy or 14 days after priming with not less than 0 1μg nor more than 1 0μg of oestrone * Only healthy mature ovariectomized mice are used Each animal receives two injections of 0 2 c c of solution with a 12 hr interval between injections Great care is taken to avoid using unhealthy animals or mice with regenerated ovaries Smears are taken with an ear curette and

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* We find no difference in residual effect of oestrone over this dosage range at 14 days or later after injection

read immediately without staining Prooestrous, oestrous, or metoestrous smear types are considered positive The first smear is taken at the time of the second injection and eight succeeding smears are taken at the rate of two per day with an interval of not less than 8 hr nor more than 16 hr between any two smears

THE STANDARD CURVES

In order to set up a standard of oestrogenesis our data have been analysed on the basis of the extent of vaginal cornification exhibited in a group of control mice receiving only olive oil and a series of animals receiving various dosages of oestrone The data on these animals are given in Table I It will be noted that although a smear is taken at the time of the second injection (i e 12 hr after the first injection) the first recorded smear is that taken at 24 hr after the first injection In fig 1 we plot the percentage of positive smears obtained at the eight recorded times of smearing in the controls and in two groups of oestrone injected animals in order to illustrate the way in which the smears change with time It will be noted that in both controls and hormone-injected animals there is an increase in positives with time Such a result of continuous smearing in ovariectomized animals has been described by Wade and Doisy (1935) and Hu and Frazier (1935) The presence of positive smears in control animals need not prevent the establishment of oestrogenic activity in compounds injected into experimental animals provided that a significant increase of cornification over the controls can be established

TABLE I SHOWING THE AMOUNT OF OESTRONE ADMINISTERED INTRAPERITONEALLY INTO MICE AND THE NUMBER OF POSITIVE SMEARS FOUND AT VARIOUS TIMES AFTER THE FIRST INJECTION

Dosage in micrograms	No of animals	Hours							
		24	36	48	60	72	84	96	108
0 00	48	6	5	5	14	14	11	14	19
0 05	10	1	1	1	0	1	5	6	4
0 07	10	2	3	3	4	4	4	8	5
0 10	15	1	2	1	7	7	10	11	9
0 13	10	1	1	6	7	6	8	8	7
0 17	10	0	0	6	7	6	7	8	9
0 25	10	1	5	9	10	9	10	9	8
0 35	10	2	7	9	10	10	10	10	7
0 50	10	3	6	10	10	10	10	10	9

Since one of our objectives is to obtain some indication of the time course of action of presumable oestrogens we can divide our data into any grouping which will afford a usable basis of comparison Accordingly we have divided the data into two groups A = the first four recorded smears (hours 24–60 inclusive), B = the second four (hours 72–108) The data so arranged are presented in Table II It should be noted that the control animals during the A period exhibit a higher percentage of positive smears than the group receiving the lowest oestrone dosage We suggest that this may be due to a "residual" cornification in the unstimulated vagina of the controls which a low stimulating level of hormone causes to disappear by maintaining the vaginal epithelium in a pre-cornified state not sufficiently

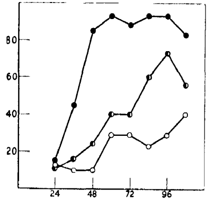

FIG 1 Abscissa time in hours after the first injection of oestrone Ordinate percentage of positive smears ○ = data on controls, ◐ = combined data on mice receiving 0 05–0 13µg , ● = combined data on mice receiving 0 17–0 50µg (see Table I)

TABLE II SHOWING OESTRONE ADMINISTERED AND THE PERCENTAGE OF POSITIVE SMEARS DURING THE A AND B PERIODS

Dosage in micrograms	A % positive	B % positive
0 00	15 6	30 2
0 05	7 5	40 0
0 07	30 0	52 5
0 10	18 3	61 7
0 13	37 5	72 5
0 17	32 5	75 0
0 25	62 5	90 0
0 35	70 0	92 5
0 50	72 5	97 5

advanced to be called positive by our criteria In any event when we plot the percentage positive against dosage for the A period (fig 2) the lowest oestrone dosage value falls on the smooth curve drawn through the data In fig 2 we plot also the data for the B period using log [moles × 10^{-11}] oestrone as the abscissa, because we wish eventually to compare the oestrogenic activity of oestrone with other oestrogens on a molar basis

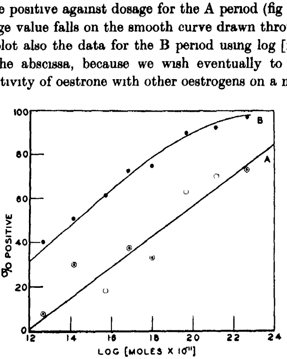

FIG 2 Abscissa oestrone dosage in log [moles × 10^{-11}] Ordinate percentage of positive smears (see Table II and text)

In order to determine whether a given compound has the same time course of action as oestrone the percentage positive read off on curve *A* should give the same oestrone molar value as the percentage positive read off on curve *B* Thus substance K (Table III) in 20μg dosage gives 35 % positive for the A period which is read off the smooth curve as equivalent to 48 8 × 10^{-11} moles of oestrone, for the B period the percentage positive is 60 % equivalent to 35 6 × 10^{-11} moles of oestrone If the difference between 48 8 × 10^{-11} and 35 6 × 10^{-11} moles is significant then K in this case would be judged to show a time course of action unlike that of oestrone, if the difference is not significant its time course of action is judged to be the same as oestrone A measure of the significance of this difference is obviously obtainable from the scatter of the oestrone injected groups of five about the curves For this purpose we have taken the fifteen groups of five animals injected with the various oestrone dosages and determined for each group the deviations from curves *A* and *B* Then, if Δ_a, Δ_b are the differences (true dose) − (dose read from the curve), we calculate the standard deviation of the difference between the readings from curves A and B by the formula

$$\sigma^2_{\text{diff}} = \frac{\Sigma\Delta_a^2 + \Sigma\Delta_b^2 - 2\Sigma\Delta_a\Delta_b}{14}$$

We use this formula because Δ_a and Δ_b are determined on the same animals, and hence may be expected to be correlated In applying this standard deviation to substances other than oestrone we regard differences of less than 3σ as not established as significant For these data $\sigma_{\text{diff}} = 13\ 61 \times 10^{-11}$ moles Therefore, in the example cited, the difference in molar value for K on the A and B curves is $48\ 8 \times 10^{-11} - 35\ 6 \times 10^{-11} = 13\ 2 \times 10^{-11}$ less than σ_{diff} and not significant We deduce therefore that K has the same time course of action as oestrone The value of σ_{diff} is of course decreased if more than a single group of five animals is employed, it obviously becomes $\sigma/\sqrt{N}$, where $N =$ the number of groups of five test animals We express the potency of a given compound as a percentage of oestrone potency by averaging the A and B equivalents and taking this average as a percentage of the injected molar dosage of the compounds * Thus compound K in $20\mu g$ dosage has an assay value in mole $\times 10^{-11}$ of oestrone of 42 2, it was injected in a dosage of 6260×10^{-11} moles $\frac{42\ 2}{6260} = 0\ 67\ \%$, i e K has 0 67 % the activity of oestrone

Since the control animals give a certain percentage positive for both the A and B periods (Table I) we have to determine the percentage positive which is significantly different from the controls in order to denominate a given substance as oestrogenic at all The task of determining this difference is complicated by the fact that in a low dosage on the A curve oestrone gives a lower percentage positive than the controls If we take nine groups of five animals in the controls and determine the "equivalent" oestrone dosages for the A and B curves we get values for the A curve which are "too high" In determining the significant level above these we accordingly are bound to set a level which is weighted against any weakly oestrogenic action Nevertheless we perform our calculations on this basis since we desire to be as rigorous as possible Our procedure then

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* Of the seventeen groups of five mice used in the oestrone injections the observed value of percentage positive for curves A and B combined never departed from the expected value by more than 15 %, usually much less We cannot, however, decide the theoretical maximum possible error without a more elaborate statistical analysis than the data warrant For this reason the values of the last column of Table III must be taken as approximations, the degree of variability of which cannot be completely defined

TABLE III THE DATA OBTAINED BY ASSAY OF THE OESTROGENIC SUBSTANCES AND CONTROLS

Substance code letter	Dose in micrograms	Mole × 10^{-9}	% positive for curve A	Equivalent value mole × 10^{-11} of oestrone	% positive for curve B	Equivalent value mole × 10^{-11} of oestrone	Significant level of diff in time course from oestrone*	Assay value in mole × 10^{-11} of oestrone	% activity of oestrone
A	0 1	0·276	6 2	18 7	50	26 9	1 16	22 8(?)	82 6
	0 3	0 828	55	94 8	95	31 8	1 064	113 3	136 8
B	0 1	0 369	35	48 8	50	26 9	1 90	37 85	102 6
	0 15	0 554	65	132 4	75	55 7	2 76	94 1	170·0
	0·2	0·738	55	94 8	100	110 0	3 10	—	—
C	0 2	0 694	45	68 4	80	65 5	0 14	66 95	96 5
	0 3	1 041	65	132 4	65	41 1	3 74	—	—
	0 4	1 388	65	132 4	70	47 9	3 25	—	—
D	50	313	40	57 5	65	41 1	1 07	49 30	0·16
E	50	181	20	29 7	25	13 8	2 44	21 75(?)	0 12
F	50	313	45	68 39	55	30 9	2 54	49 65(?)	0·16
	75	469	40	57 5	75	55 7	0 10	56 60	0 12
G	25	92	30	41 3	60	35 6	0 47	38 45	0·42
H	25	79	30	41 3	75	55 7	0 96	48 5	0·61
I	50	204	25	35 0	55	30 9	0 40	32 95(?)	0 16
J	50	204	25	35 0	35	17 6	2 19	26 30(?)	0·25
K	10	31 3	35	48 8	50	26 9	1 89	37 85	1 21
	20	62 6	35	48 8	60	35 6	1 00	42 2	0 67
	35	110	50	80 4	75	55 7	1 17	68 05	0 62
	50	157	60	112 2	80	65 5	1 72	88 85	0·57
L	50	184	40	57 5	70	47 9	0 59	52 7	0·29
M†	10	34 7	40	57 5	75	55 7	0 10	56 6	1 63
	15	52 1	35	48 8	64 7	41 1	0 54	44 95	0·86
	25	86 8	75	184 5	95	131 8	1 07	158 15	1 82
	30	104 1	50	80 4	80	65 5	0 65	72 95	0 70
N	20	70	15	25 1	75	55 7	2 54	40·4	0 58

O	60	121	35	48 8	45	23 3	2 35	36 05	0-30
P	25	67 3	30	41 3	40	20 2	2 28	30 75(?)	0 46
Q	50	269	25	35 0	20	—	—	—	—
R	50	250	30	41 3	50	26 9	1 37	34 1	0-14
S	50	236	10	21 3	45	23 3	0-29	22 3(?)	0-09
T	50	167	30	41 3	55	30 9	0-92	36 1	0-22
	100	333	30	41 3	60	35 6	0 47	38 45	0-12
U	50	170	30	41 3	60	35 6	0 47	38 45	0-23
V	25	128	20	29 7	45	23 3	0 76	26 5(?)	0-21
	50	255	35	48 8	65	41 1	0-54	44 95	0 18
	75	383	25	35 0	85	79 1	2 602	57 05	0-15
	100	510	35	48 8	85	79 1	1 544	63 95	0 13
W	50	207	50	80 4	44	23 3	4 83	51 85	0-25
							(not significant—4 animals)		
X	100	402	0	15 78	20	—	—	—	—
	200	803	0	15 78	15	—	—	—	—
Y	100	405	0	15 78	—	—	—	—	—
Z	50	201	5	17 95	10	—	—	—	—
	200	409	5	17 95	20	—	—	—	—
A1	100	301	40	57 5	20	—	—	—	—
B1	100	318	15	25 1	5 0	—	—	—	—
C1	100	333	40	57 5	20	—	—	—	—
D1	100	355	30	41 3	20	—	—	—	—
E1	100	305	10	21 3	20	—	—	—	—
	1000	3050	15	25 1	20	—	—	—	—
F1	100	382	10	21 3	20	—	—	—	—
G1	Oestrone choleic acid								
	0 2	—	30	41 3	60	35 6	0 47	38 45	—

* Obtained by dividing Δ of log mole equivalent for curves A and B by standard error of computation S E = 0 134

(?) Not significantly different from controls (see text)

† The 10 and 25 μg doses were injected shortly after the preparation, the 15 and 30 μg doses later when the melting point had lowered

is to read off the oestrone equivalents for the nine groups of five controls on curves *A* and *B* and then to take the mean of these two values The mean of these means is 21 84 $\times 10^{-11}$ moles, its $\sigma = 4\ 65 \times 10^{-11}$ and we take 2 5σ as the significant value (this is approximately the value that would be required by Fisher's *t* test) Thus any compound giving a mean oestrone molar value of 33 93 $\times 10^{-11}$ or greater is considered oestrogenic, i e it exhibits a percentage positive significantly higher than the controls

THE TEST COMPOUNDS

In Table III we list the series of substances injected intraperitoneally with the dosage injected, the equivalent oestrone values, and the activity of each expressed as a percentage of oestrone activity These compounds include (1) native oestrogens in the form of oestrone monobenzoate (A), oestradiol (B), oestriol (C), (2) a variety of phenanthrene and hydrophenanthrene derivatives (G to P and U to F1), (3) a set of diphenyl derivatives (Q to T), and (4) three naphthalene derivatives (D to F) The structural formulae of these compounds and their code letters are listed in Table IV, and their names and melting points in Table V

Definite oestrogenic activity is shown in the dosages employed by certain of the substances in each of the three groups of synthetic compounds The compounds which we designate as inactive are divisible into two groups (1) those giving a sufficient percentage positive during the B period to fall upon our standard curve (E, I, J, P, R) and (2) those which show a percentage positive during the B period which is below the lowest point on the standard curve (X to F1) We have given oestrone equivalent values for the former group even though they do fall within the range of our control group It should be noted that these compounds *may* be oestrogenic in higher dosage P, for example, is the diacetate of M, it was injected in 25μg dosage in order to compare its activity with that of M on the one hand and O (the dibenzoate of M) on the other, O in 50μg dosage is significantly oestrogenic by our criteria, and there is no reason to suppose that in increased dosage P would not be about as potent as O, since the acetate of native hormones ordinarily is about as active as the benzoate

Of the synthetic compounds showing definite activity we can state that their time course of action is never different from that of oestrone (see column 8 Table III) * This has some interesting implications that will be considered in the discussion Further generalization is difficult We can discover no very clear association between structure and activity The presence of auxiliary hydroxy or methoxy groups upon the 1st ring* of the phenanthrene nucleus seems to enhance the activity of the phenanthrene derivatives In fact, were it not for V we should be inclined to think that the presence of such an auxiliary group is necessary for activity, V has been found active by other workers (Cook, Dodds, Hewitt and Lawson 1934, also Butenandt 1936) Of course we could amend the rule to include any phenanthrene nucleus having either an auxiliary hydroxy or methoxy group on the 1st ring or a ketonic saturated ring, but the variety of phenanthrene derivatives tested is not sufficient to allow such statement It is certain that the phenanthrene nucleus is not necessary for oestrogenic activity as the data on the naphthalene and diphenyl derivatives attest (cf Dodds 1936, Robson and Schonberg 1937) Somewhat enhanced activity of the dicarboxylic anhydrides is apparently attained by increasing the auxiliary hydroxy and methoxy groups (compare G and H, L, M and N) Benzoylation (O) or acetylation (P) of M does not enhance its activity The apparent decrease on a molar basis appears to be due in part to the addition of the inactive benzoyl and acetyl groups and probably also to a slightly lower rate of absorption of these compounds

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* The one compound, W, which gives an apparently significant value of $\frac{\text{diff}}{\sigma_{\text{diff}}}$ (4 83) was tested on only four animals and cannot, therefore, be considered validly different

Of the three forms of native oestrogens tested oestrone benzoate (A) shows no difference in time course of reaction from oestrone whereas increasing the dosage of oestradiol (B) and oestriol (C) results in an increasing divergence between their time course and that of oestrone such that obviously significant differences are attained in the highest dosages employed (column 8, Table III) For these dosages, where $\frac{\text{diff}}{\sigma_{\text{diff}}}$ is greater than 3 0, we therefore do not calculate activity in terms of oestrone In the low dosages where comparison is permissible no obvious difference exists between the extent of their activity and that of oestrone, this appears to be true also of oestrone benzoate It should be noted that wide differences in potency have been reported in assays conducted by subcutaneous injection

SUBCUTANEOUS INJECTIONS

Five sets of mice were injected with oestrone subcutaneously in the dosages listed in Table VI In the four lower dosage groups there is no

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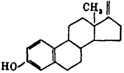
* We call the benzene nucleus correspondent to the unsaturated ring of oestrone the 1st ring

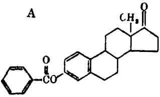
TABLE IV

Oestrone



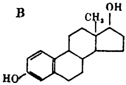
% Activity = 100

A



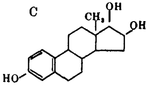
% Activity = 109·2

B



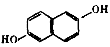
% Activity = 102·6

C



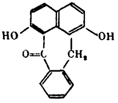
% Activity = 96·5

D



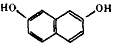
% Activity = 0·16

E



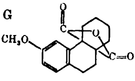
% Activity = 0·12

F



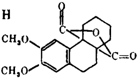
% Activity = 0·14

G



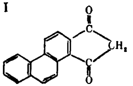
% Activity = 0·42

H



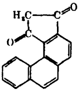
% Activity = 0·01

I



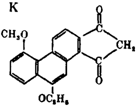
% Activity = 0·16

J



% Activity = 0·25

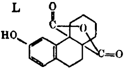
K



% Activity = 0·77

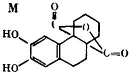
TABLE IV (*continued*)

L



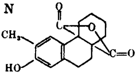
% Activity = 0·29

M



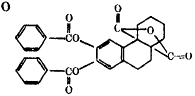
% Activity = 1·72

N



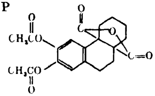
% Activity = 0·58

O



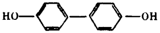
% Activity = 0·30

P



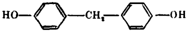
% Activity = 0·46

Q



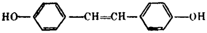
% Activity = 0·00

R



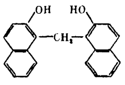
% Activity = 0·14

S



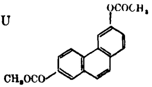
% Activity = 0·09

T



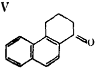
% Activity = 0·17

U



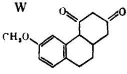
% Activity = 0·23

V



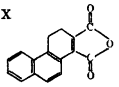
% Activity = 0·19

W



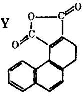
% Activity = 0·25

X



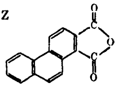
% Activity = 0·00

Y



% Activity = 0·00

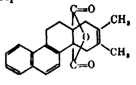
Z



% Activity = 0·00

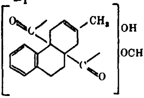
TABLE IV (*continued*)

A₁



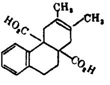
% Activity = 0 00

B₁



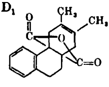
% Activity = 0 00

C₁



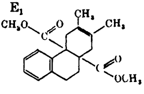
% Activity = 0 00

D₁



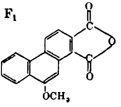
% Activity = 0 00

E₁



% Activity = 0 00

F₁



% Activity = 0 00

TABLE V NAMES AND MELTING POINTS OF COMPOUNDS DESCRIBED IN TABLES III AND IV

		Made by (initials)
A	Oestrone benzoate	
B	Oestradiol	
C	Oestriol	
D	2, 6 Dihydroxynaphthalene	L F F
E	Dihydroxypleiadone	L F F
F	2, 7 Dihydroxynaphthalene	L F F
G	6 Methoxy 1, 2, 3, 4 9, 10, 11, 12 octahydrophenanthrene 11, 12 dicarboxylic anhydride	E B H
H	6, 7 Dimethoxy 1, 2, 3, 4, 9, 10, 11, 12 octahydrophenanthrene 11, 12 dicarboxylic anhydride	E B H
I	1′, 3′ Diketo 1, 2 cyclopentenophenanthrene M P 240–5° (dec)	E B H
J	Phenanthrene 3, 4 dicarboxylic anhydride	M F
K	5 Methoxy 9 ethoxy 1′, 3′ diketo 1, 2 cyclopentenophenanthrene M P 207–8° corr	E B H
L	6 Hydroxy 1, 2, 3, 4, 9, 10, 11, 12 octahydrophenanthrene 11, 12 dicarboxylic anhydride	E B H
M	6, 7 Dihydroxy 1, 2, 3, 4, 9, 10, 11, 12 octahydrophenanthrene 11, 12 dicarboxylic anhydride M P 147 5–148 5° corr	E B H
N	6 Methyl 7 hydroxy 1, 2, 3, 4, 9, 10, 11, 12 dicarboxylic anhydride M P 134–6°	E B H
O	Dibenzyl derivative of M M P 175–175 5° corr	E B H
P	Diacetyl derivative of M M P 151 5–152° corr	E B H
Q	4, 4′ Dihydroxydiphenyl—activity observed by Cook and Dodds	L F F

TABLE V (*continued*)

		Made by (initials)
R	4, 4′-Dihydroxydiphenyl methane—activity observed by Cook and Dodds	L F F
S	4, 4′-Dihydroxy stilbene	L F F
T	Di 2 naphthol methane	L F F
U	2 6 Diacetoxy phenanthrene	L F F
V	1 Keto 1, 2, 3, 4 tetrahydrophenanthrene M P 95 5° Activity first determined by Cook and others	M F & E B H
W	2, 4 Diketo 6 methoxy 1, 2, 3, 4, 9, 10, 11, 12 octahydrophenanthrene M P 150 5–151°	W P C
X	3, 4 Dihydro 1 2 phenanthralic anhydride M P 254–6°	E B H
Y	1, 2 Dihydro phenanthrene 3, 4 dicarboxylic anhydride M P 150°	M F
Z	1, 2 Phenanthralic anhydride M P 309–10°	E B H
A1	2, 3 Dimethyl 1, 4, 11, 12, 13, 14 hexahydrochrysene 13, 14 di carboxylic anhydride M P 196–196 5°	E B H
B1	1, 4, 9, 10, 11, 12 Hexahydro 2 3 dimethylphenanthrene 11, 12 dicarboxylic monomethyl ester M P 155–7°	E B H
C1	1, 4, 9, 10, 11, 12 Hexahydro 2 3 dimethylphenanthrene 11, 12 dicarboxylic M P 176–7° corr	E B H
D1	1, 4, 9, 10, 11, 12 Hexahydro 2, 3 dimethylphenanthrene 11, 12 dicarboxylic anhydride M P 95–6°	E B H
E1	1, 4, 9, 10, 11, 12 Hexahydro 2, 3 dimethylphenanthrene 11, 12 dicarboxylic dimethyl ester M P 93 5–94° corr	E B H
F1	9 Methoxyphenanthrene 1, 2 dicarboxylic anhydride M P 241–2°	E B H
G1	Oestrone choleic acid—a molecular compound of oestrone and choleic acid Its structure is unknown Determinations of the ratio of choleic acid to oestrone gave values ranging from 4 to 1 to 7 to 1 Although the exact ratio of activity is not known, on a molecular basis the oestrone in this molecule must be at least 4 and possibly 7 times as active as free oestrone	M S N

The initials in this table refer to the following persons who prepared the compounds to which their initials are subscribed

L F F = Dr Louis F Fieser
M F = Mrs Mary Fieser
E B H = Dr E B Hershberg
W P C = Dr W P Campbell
M S N = Dr Melvin S Newman

significant difference between the percentage positive in the A and B periods, only with the 1 0μg dosage the B period exhibits a significantly higher percentage positive than the A period ($\chi^2 = 6\ 14$, $P = 0\ 015$) In this respect subcutaneous injections differ markedly from intraperitoneal injections of oestrone The increase in percentage positive with a given increment of increase in hormone dosage is roughly one-third to one-half that obtained with intraperitoneal injection, furthermore with intraperitoneal injection the effect increases logarithmically with dosage over a considerable range (see fig 2), whereas the subcutaneous injection produces an effect which is roughly directly proportional to the dosage over the range employed We note that the lowest subcutaneous dosage employed (0 02μg) gives sufficient positives for it to be considered significantly different from the controls[*] Furthermore, the lower dosages in subcutaneous injection give higher percentages positive during the A period than corresponding intraperitoneal dosages

TABLE VI THE PERCENTAGE OF POSITIVE SMEARS DURING THE A AND B PERIODS IN MICE RECEIVING OESTRONE SUBCUTANEOUSLY, FIVE MICE IN EACH DOSAGE GROUP

Dosage in micrograms	A % positive	B % positive
0 02	45	40
0 04	35	60
0 085	60	55
0 30	55	55
1 00	55	90

TABLE VII THE RESULTS OF THE SUBCUTANEOUS INJECTION OF THREE SYNTHETIC COMPOUNDS AND OF OESTRONE CHOLEIC ACID, FIVE MICE IN EACH DOSAGE GROUP

Substance	Dosage in micrograms	A % positive	B % positive	Level of difference in time course from oestrone injected intraperitoneally
K	450	15	50	0 22
M	700	20	30	2 15
M	2000	25	50	0 83
M	7000 (3 inj)	30	35	2 72
V	7000	10	25	1 38
Oestrone choleic acid	0 1	10	25	1 38
,,	0 2	25	45	1 29
,,	0 4	85	90	3 06

We have injected subcutaneously three synthetic compounds (K, M, and V) and oestrone choleic acid These data are presented in Table VII K in 450μg dose and M in the two higher dosages employed give percentages positive significantly higher than the controls, whereas V does not in 7000μg dose V has been found oestrogenic in mice by others (cf Butenandt and Schramm 1936, Thayer, MacCorquodale and Doisy 1937) in doses of 25 mg and above upon subcutaneous injection The oestrone choleic acid in 0 1μg dose gives a result not significantly different from the controls, but does in the 0 2μg and 0 4μg doses The fifth column of Table VII indicates that none of the compounds listed differs significantly in its time course of action from oestrone injected intraperitoneally except oestrone choleic acid in the highest dosage Since this dosage gives a percentage positive in the A period which must be determined from our A curve by extrapolation, the value 3 06 $\left(\text{of } \frac{\text{diff}}{\sigma_{\text{diff}}}\right)$ is open to question, as the curve would presumably become of lesser slope with high percentages positive

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[*] We employ the forty eight animals of Tables I and II as controls for the sub cutaneous injections also since the subcutaneous injection of olive oil does not cause any significant difference in the vaginal smears The A and B period data are compared with the control data using the χ^2 test to determine significant differences

DISCUSSION OF THE DATA ON OVARIECTOMIZED MICE

We have indicated previously (Pincus and Werthessen 1936) why we consider advisable the intraperitoneal injection of the types of synthetic compound employed Their relative insolubility makes for slow absorption from the subcutaneous site and furthermore we have noted positive inflammatory reactions against the materials consisting of lymphatic blockage, capillary breakdown, and walling off of the oil globule by connective tissue The more open lymphatic drainage of the peritoneum permits fairly rapid entry of the solutions into the circulation although eventually even these vessels would presumably exhibit typical inflammatory reactions against stimulating materials

When, indeed, we come to consider the relative potencies of the various substances the possibility of not merely slow diffusion into the circulation but also of actual bodily reactions preventing their entry becomes of considerable importance For even if no acute inflammatory reaction against them is observable more subtle events in the lymph and capillary networks at the site of absorption may be most effective (see Menkin 1936) In considering the work of Cook, Dodds and their collaborators we were struck by the fact that relatively large subcutaneous doses of most of the synthetic compounds they employed were necessary to induce an oestrus response, e g 10–100 mg This may be due to the fact that they do indeed possess a low potency or that they enter with difficulty into the circulation On the latter basis doses up to a certain level are ineffective either because of extreme insolubility or the ability of the inflammatory mechanism to prevent absorption of irritating substances, whereas high dosages even with a low solubility deliver sufficient quantities to permit detectable activity or are so large that the inflammatory mechanism cannot completely cope with all the "invading" materials and some manages to get through into the circulation That slow absorption due to either of these mechanisms is a factor is indicated by the fact that certain of the compounds employed by Cook, Dodds and others continue to be active for 2–3 weeks after the initial injections They find, indeed (Cook, Dodds, Hewitt and Lawson 1934) that V in 100 mg dosage in rats is ineffective if delivered in three consecutive subcutaneous injections on the same day, whereas the same amount given in three injections on three consecutive days gives a positive oestrus response lasting 20 days They explain this difference on the grounds that rapid absorption takes place during the first 12 hr after injection, falling to a slow steady level, and, since more oestrogenic activity is needed to initiate oestrogenic activity than to maintain it, the 1 day injections deliver a large, but insufficient, amount over too short a period of time to initiate oestrus, the 3 day injections deliver successive peaks of material which are cumulatively sufficient to overcome the barriers to oestrus initiation and the subsequent slow absorption is sufficient to maintain oestrus

Our data indicate that not only the rate of absorption but that the amount absorbed is also an important factor For by intraperitoneal injection V is definitely oestrogenic in 50μg dosage, whereas in 7000μg dosage subcutaneously the degree of vaginal stimulation obtained is not significantly indicative of oestrogenic action Furthermore, M is active in 10μg dose intraperitoneally but not active in 700μg dosage by subcutaneous injection and is definitely active in 2000μg dosage subcutaneously Increasing M to 7000μg in subcutaneous injection scarcely increases the percentage of positive smears whereas increasing the intraperitoneal dosage does raise the percentage positive in roughly the same manner as with oestrone The conclusion is inescapable that only a certain small proportion of the material injected subcutaneously actually gets into the circulation, and certainly a much larger proportion of the intraperitoneally injected material is active It is apparent that the amount of active material leaving the subcutaneous site varies from compound to compound We can set the minimum effective dosages roughly for K, M and V as respectively 8μg , 7μg , and 40μg , by subcutaneous injection they are approximately 450μg , 2000μg , and more than 7000μg , the ratios of the two are 1 56, 1 286 and 1 175+

Oestrone itself presents a curious contrast to the synthetic compounds In the lower dosages subcutaneous injection appears to be much more effective We believe the explanation for this lies in the way the vaginal stimulation is effected in the two cases Intraperitoneal injection by our method we believe presents a close imitation of what happens during a normal cycle where we have a period of epithelial growth with slight cornification during the prooestrum and oestrum, and a period of marked desquamation and leucocytic infiltration in the metoestrum The latter we conceive of as being due to a withdrawal of the hormone stimulation, the former as due to positive stimulation By intraperitoneal injection we obtain a rapid stimulation giving in the lower dosages prooestrus types of growth often scarcely detectable by our method of classification and fairly rapid withdrawal of the stimulus giving typical postoestrus symptoms The subcutaneous injection is effective however by virtue of a continuous stimulation with a latent period of roughly 48–60 hr between injection and initial effect and a protraction of this effect with a falling off as the absorbable oestrone declines This appears to us to be the basis of the difference in time course of action of oestrone administered by the two routes We get a lower percentage positive with low doses of oestrone injected intraperitoneally because the rapid absorption is accompanied by fairly rapid excretion and the mild prooestrus growth is scarcely identifiable during the A period During the B period the smears are more easily identified as positive and so the percentages positive more nearly approach those observed in subcutaneously injected animals, but they do not quite attain the subcutaneous levels because of the excretion of active material This is borne out when we consider more closely what happens during the A period in the two cases The percentages positive for the three lowest intraperitoneal dosages for the 24th and 36th hour smears combined and the 48th and 60th hour are respectively 7 1 and 11 4% which are in the ratio of 1 1 6, for the three lowest subcutaneous dosages the corresponding percentages are 26 7 and 73 3 which are in the ratio of 1 2 7

The synthetic compounds injected subcutaneously show no significant difference in time course of action from oestrone injected intraperitoneally presumably because only a certain small but stimulating amount of the material escapes at the time of injection and then no more, or at least insufficient to continue stimulation Oestrone choleic acid shows the same time course of action as oestrone by both intraperitoneal and subcutaneous injection presumably because the attachment of choleic acid radicals to the oestrone molecule makes it more rapidly absorbable so that the period of absorption of active material is much shorter than that of oestrone It is obvious also from the data of Table VII that doubling the dosage of oestrone choleic acid much more than doubles its activity We can only deduce that the choleic acid addition not only makes the molecule more easily absorbed but also prevents its destruction It is notable in this connexion that oestrone choleic acid is the only compound that exhibits identical activity by both routes of administration Unfortunately we do not know the molecular weight of the oestrone choleic acid compound and so cannot give its activity in terms of oestrone We regret also having no data on the results of injecting A, B, and C subcutaneously, particularly since B and C in the higher dosages used exhibit obviously significant differences in time course of action from oestrone

Although, by intraperitoneal injection we have succeeded in enhancing the activity of the synthetic compounds presumably by increasing the effective amount absorbed we cannot state that we have thereby determined their "real" activity For we do not know how much material actually does work in stimulating vaginal growth Intraperitoneal injection does not necessarily mean complete absorption, nor do we have any notion of the rate of excretion or destruction of these compounds For this reason we cannot examine too closely the relation between structure and activity We are inclined to agree with Dodds (1936) that these compounds may be likened to active degradation products of the oestrone molecule, especially since their time course of action is so uniformly like that of oestrone But a number of the substances might equally well be regarded as degradation products of oestradiol or oestriol Now oestradiol differs significantly in time course of action from oestrone only in the highest dosage employed (0 2μg) and the difference resides in the fact that during the B period 100 % positive smears were obtained, this demanded determination of the oestrone equivalent value by extrapolation of curve *B* and is therefore open to question But oestriol is different from oestrone at a justifiably measurable level (see Table III) and the difference is due to relatively low percentages positive during the B period This would indicate that the stimulating effect of oestriol falls off more rapidly than that of oestrone The mouse accordingly has a somewhat more efficient mechanism for excreting (or destroying) oestriol than oestrone On this basis we can unify all our experimental results by assuming that the synthetic compounds are eliminated as slowly as or more slowly than oestrone If they are eliminated more slowly than oestrone the falling off in effect exhibited by oestrone at the 108th hour (see fig 1) would not occur, but since the falling off in the oestrone series is of such a degree as not to affect markedly the percentage positive for the whole B period these compounds would be oestrone-like in their time course Thus K (which, incidentally, has a closed 4th ring) is very much like oestrone in that the percentage positive (all doses combined) is 50 0 at the 108th hour compared with 71 7 % during the rest of the B period, M and V show respectively 89 4 and 100 % positive at the 108th hour compared with 75 9 and 71 1 % during the rest of the B period We have, in fact, observed some evidences of continued stimulation by M as late as a month after its injection

We are mindful that certain of the compounds here found to be active may be oestrogenic only in the sense that they stimulate the production of a certain degree of vaginal activity and that they may not assume all the activities exhibited of the native hormones As a preliminary test of this notion we injected intraperitoneally into ovariectomized mice three of the synthetic compounds in combination with colchicine after the manner described by Allen, Smith and Gardner (1937) and counted the number of mitoses per uterine cross section (cut 10μ thick) and per vaginal longitudinal section The data are presented in Table VIII

TABLE VIII THE MEAN NUMBER OF MITOSES PER UTERINE CROSS SECTION AND PER VAGINAL LONGITUDINAL SECTION IN VARIOUS GROUPS OF OVARIECTOMIZED MICE

Compound injected	Amount injected in micrograms per mouse	No of animals	Mitoses per animal in uterine mucosa	Mitoses per animal in vaginal epithelium
Control	—	4	25 0	8 0
F	140	4	31 2	73 5
K	58	4	69 0	34 8
M	170	3	48 3	40 3

It is apparent that whereas all the compounds give obvious stimulation of vaginal activity only K differs markedly from the control in increasing mitoses in the uterine endometrium, and M problematically so While these data cannot be taken as definitive they do indicate that the compound most remote from the hormones in structure, F, exerts its chief effect upon the vaginal epithelium It may then be considered a "pseudo-oestrogen" From the point of view of the relation between structure and activity this distinction is a very important one It may be that the vaginal epithelium cornifies in response to any ring compound having one or more of several possible auxiliary groups whereas other forms of oestrus activity are more specifically controlled We have therefore conducted the rabbit experiments detailed below

EXPERIMENTS ON OVUM GROWTH AND ENDOMETRIAL PROLIFERATION IN THE OVARIECTOMIZED RABBIT

The technique employed has been previously described (Pincus and Werthessen 1937), each rabbit doe received a given (or no) dosage of progesterone in six divided subcutaneous doses beginning the day after ovariectomy (38–40 hr after copulation) with the simultaneous intraperitoneal injection of the various test compounds, the animals were sacrificed at 18–20 hr after the last injection, the ovum diameters measured, and the uterus fixed for subsequent sectioning and measurement In Table IX we list the various combinations of progesterone and oestrogens and the data on ovum size and endometrial growth For any given

TABLE IX THE EFFECTS UPON OVUM SIZE AND UTERINE PROLIFERATION (G/M) OF VARIOUS OESTROGENIC SUBSTANCES

Rabbit	Amount of progesterone injected (mg)	Oestrogenic substance injected	Amount of oestrogen injected (mg)	Mean egg diameter (μ)	Mean G/M
P 0–1	0 42	A	**0-003**	377	0 361
P 0–4	0 42		**0 010**	556	**0 629**
P 0–5	0 42		0 050	*205*	0 382
P 0–22	0 42		0 075	*229*	**0 294**
P 0–29	0 42	B	0 003	**828**	**0 703**
P 0–23	0 42		0 005	—	**0 648**
P 0–25	0 42		0 020	—	0 476
P 0–28	0 42		0 075	**828**	0 353
P 0–24, 31	0 42	C	0 025	**900**	**0 705**
P 0–26	0 42		0 250	—	0 541
P 0–30	0 42		0 500	**754**	*0 230*
P 0–21	0 42	V	50 0	453	**0 644**
P 0–13	0 42		100 0	*151*	*0 104*
P 0–18	0 42		100 0	*221*	0 505
P 0–6	0 42	M	10 2	540	**0 663**
P 0–14	0 42		50 0	460	**0 789**
P 0–8	0 42		54 0	741	0 564
P 0–19	0 42		100 0	*188*	**0 671**
Q	0 00		60 0	—	0 281
P 0–17	0 42	K	75 0	410	**0 645**
P 0–15	0 42		300 0	629	**0 676**
P 0–10	0 42		300 0	**890**	**0 697**
P 0–16	0 00		300 0	193	0 282
P 0–7	0 42	R	40 0	615	0 449
P 0–9	0 42		205 0	—	0 561
P 0–11	0 42		450 0	524	0 516

progesterone dosage we determine from our standard curve (Pincus and Werthessen 1937) the expected ovum size and mucosal growth, and employing the standard deviation of the standard curve can determine whether any given set of measurements deviates significantly from expectation A significant decrease from expectation is indicated in the table by black type, a significant increase by italic type The progesterone dosage used (0 42 mg) should in a single rabbit give a mean ovum diameter of 500 ± 125 5 μ, and a G/M value* of 0 460 ± 0 0776 The lowest dosage of oestrone ($3\mu g$) injected with the progestin has no significant effect upon either ovum diameter or endometrium development But the $10\mu g$ dosage of this compound and the lowest dosage of all the others (except R) give in each instance a significant increase of G/M, e g an enhancement of the progesterone effect On the face of it this would seem to indicate the familiar synergistic activity of oestrin and progestin But increasing the dosage of oestrone, B, C, and V result in a reduction of glandular proliferation whereas increasing the dosages of M and K continues to give marked enhancement of the progesterone effect This might be explicable on the grounds that M and K are progestational compounds, but when these are injected as substitutes for progesterone, i e animals Q and P 0–16, they do not act as progestins

We can unify these results hypothetically on the basis of the presumed fate of these compounds after injection Schematically presented it is as follows

$$\begin{tikzcd}
 & \text{excretion} & \\
S \arrow[ru] \arrow[r] \arrow[rd] & D \arrow[r] & \text{excretion} \\
 & C \arrow[r] \arrow[d] & \text{excretion} \\
 & D' \arrow[r] & \text{excretion}
\end{tikzcd}$$

in which S = the hormone, or synthetic compound, D any (and all) inactive degradation or "destruction" product, C any (and all) active converted form of the hormone, and D' any (and all) inactive degradation product of C We present as entirely reasonable the postulate that the inhibition of any one of these processes will result in the accumulation of the products of the preceding reactions Thus if D represents an inactive breakdown product of progesterone the prevention of its excretion will cause not only its accumulation but also the accumulation of progesterone through a decrease in the rate of the reaction $S \rightarrow D$ (This is essentially the postulate underlying mass action kinetics) Progesterone effects will then be enhanced unless the excretion of progesterone or its conversion to another compound is increased proportionately Fels (1935) has in fact reported that progesterone activity in the Allen-Corner test is enhanced by the simultaneous injection of pregnandione which is itself inactive Pregnandione might very well be a natural degradation product of progesterone, its structural configuration, in any event, makes it seem likely that the same enzyme system which is concerned in the conversion of progesterone might act upon the pregnandione molecule The synergistic effect of the low dosages of the compounds of Table III can be explained by assuming that of the presumable enzyme systems concerned in the conversion and degradation of the ovarian hormones some can act upon all the various compounds related to the hormones Thus the enhancement of the progesterone action by low dosages of oestrone, oestradiol, and oestriol may be due to the fact that the oestrogens are destroyed by the same system as progesterone and the partial saturation of this destructive system by the oestrogens prevents the normal rapid destruction of progesterone That progesterone is ordinarily rather rapidly converted seems indicated by the fact that its recovery in urine has been reported only once (Loewe and Voss 1934) and then in the concentration of 1 Allen-Corner unit per 20 l of late menstrual urine We have been unable to recover any progestin activity in the urine of ovariectomized rabbits receiving up to 6 mg of progesterone at the rate of 1 mg per day The oestrogens, on the other hand, can be recovered in part after injection (Pincus and Zahl 1937), so we judge that they have a lower "partition coefficient" for the destructive system The compounds V, M, K, and R represents presumable degradation products of the oestrogens of such a chemical nature that they still retain a certain amount of oestrogenic activity (cf Dodds 1936) They might occupy positions lower down in the degradation chain than the native hormones but nevertheless inhibit progesterone destruction in the same way as pregnandione Compound R in the dosages employed appears to be an exception since it gives no enhancement of progesterone activity yet we and Dodds (1936) have found it to have mild oestrogenic activity On our hypothesis we can only assume that this compound is not acted upon by the enzyme chain that deals with the native hormones

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* G = area of the glandular portion of the endometrium, M = total area of the endometrium

When we come to consider the effects of higher dosages it is evident that the native hormones inhibit the progestational effect and that oestriol is less effective than either oestrone or oestradiol This is the familiar antagonistic effect of the oestrogens (cf Hisaw 1932, Courrier and Raynaud

1933, Robson 1936) Of the synthetic compound only V exhibits this effect, we suggest that V alone has the proper molecular configuration for interfering with the reactions leading to glandular proliferation

These data show that ovum growth and glandular proliferation are affected differentially by these compounds and give thereby further evidence that various reaction chains are involved We have suggested (Pincus and Werthessen 1938) that the stimulating effect of progesterone upon egg and glandular growth is consonant with the notion that

$$\begin{tikzcd}
 & & \text{(2) glandular growth} \\
\text{Progesterone} \arrow[r] & \text{(1) mucosa} \arrow[ru] \arrow[rd] & \\
 & & \text{(3) ovum growth}
\end{tikzcd}$$

Thus oestrone (1) inhibits reactions at (2) and (3), apparently first affecting (3), oestradiol (B) and oestriol (C) inhibit (2) without affecting (3)—in fact by inhibiting (2) they seem to enhance the progesterone effects through (3) since the ova of oestriol and oestradiol injected animals are 50–80 % larger than expected Ketotetrahydrophenanthrene (V) acts like oestrone, and we note that it, too, is a ketone, M in high dosage presumably inhibits (3) leaving (2) unaffected, and K in high dosage causes increased ovum as well as progestational growth merely through its inhibition of progesterone destruction

It is a matter of some moment for our general argument that oestrone inhibits both egg growth and glandular proliferation, whereas B and C do not inhibit egg growth while definitely decreasing the degree of glandular proliferation It indicates that B and C are not involved in exactly the same reactions as oestrone This is further made obvious when we consider the finding of Pincus and Zahl (1937) that oestrone is converted to oestriol in the rabbit uterus Oestradiol (Pincus and Zahl, unpublished data) is converted to oestrone in the presence of the ovary and without the presence of the uterus The oestrone effect on the ova therefore is in some way involved in the step $S \rightarrow C$ of our general scheme of hormone conversion V, because of its ketone structure, may very well be converted by the same reaction system It may, on the other hand, be converted to oestrone in the body and exhibit what is roughly 1/1000th to 1/2000th the activity of oestrone merely because only a small amount of it is converted to oestrone Cook and others (1934) have advanced cogent arguments against the conversion *in vivo* of the synthetic compounds into the hormones, and we have tested this possibility by injecting 70 mg of V into a pregnant rabbit but recovered no oestrone, oestriol, or oestradiol in the urine The pregnant rabbit (Pincus and Zahl 1937) excretes about 60 % of injected oestrone or oestriol in the urine, so that if conversion to native hormone occurred the urine should have contained at least 20–50μg of hormone

We are left therefore with the conclusion that a chain or group of reaction systems acts upon or requires the participation of the hormone molecules and that at one point or other in the chain the synthetic compounds may enter The vaginal effect presumably occurs at a point in the chain wherein rather wide variation in chemical structure does not necessarily hinder activity Participation in certain of the uterine reactions on the other hand requires more specific structural configuration, the reactions involving ovum growth, for example, are unaffected by certain native hormones which do affect both uterine proliferation and vaginal activity

We are extremely indebted to Professor Louis F Fieser and his co-workers for their extraordinary generosity in supplying us with the synthetic substances described in Table V To the E R Squibb Co we express our gratitude for supplying the oestrone used in making oestrone choleic acid Dr C P Winsor rendered us invaluable assistance in the statistical analysis

Acknowledgement is here made of grants from the Josiah Macy jr Foundation and from Parke, Davis and Co , which have materially aided the work

SUMMARY

1 A method of assay for oestrogenic compounds is described Injections are made intraperitoneally into groups of five ovariectomized mice and smears are taken at 12 hr intervals up to the 108th hour after the first injection Menstruation curves for oestrone in various dosages are given, by employing the percentage positive smears of (A) the 24th–60th hours and (B) the 72nd–108th hours the distinctive time course in change of vaginal activity is described and its limits set

2 Comparison with the oestrone standard is made for four types of native oestrogen and twenty nine synthetic compounds On a molar basis, setting oestrone as 100 %, the activity of the synthetic compounds is never above 1 72 % (substance M), and ordinarily lower or nil By subcutaneous injection certain of these compounds show even lesser activity due presumably to their relatively slight absorption Their time course of action is in every instance no different from that of oestrone whereas oestriol (substance C) is definitely different in two dosages employed and oestradiol (B) presumably so in the highest dosage employed Oestrone monobenzoate

(substance A) does not differ in either percentage of activity or time course of action from oestrone on intraperitoneal injection, confirming the findings of Deansley and Parkes (1937) on their relative activity by intravenous injection in the capon

3 Wide variations in structural configuration (based upon phenanthrene, diphenyl, or naphthalene nuclei) involve roughly similar oestrogenic activities on the basis of vaginal stimulation, with a definite enhancement (doubling) of activity in the phenanthrene derivatives due to auxiliary hydroxyl groups in the 6 and 7 positions Of three compounds tested in ovariectomized mice one (K) apparently increases mitotic activity in both the uterine and vaginal epithelia whereas two others (F and M) appear to stimulate only vaginal mitosis

4 The simultaneous injection of certain oestrogens (except R) with progesterone into rabbits ovariectomized 18–20 hr after fertile copulation results in an enhancement of the progesterone effect upon uterine proliferation (and to some extent upon egg growth) in the lowest oestrogen doses employed In higher dosages oestrone and 1-keto-1, 2, 3, 4-tetrahydrophenanthrene (V) inhibit ovum growth and uterine proliferation, B and C inhibit only uterine proliferation and enhance ovum growth, M and K continue to enhance uterine growth (M may inhibit ovum growth in 100 mg dose), but are not progestins, R affects neither process in doses up to 450 mg The enhancement of the progesterone effect is explained on the assumption that these compounds enter into reactions the net effect of which is to prevent progesterone destruction It is deduced that the failure of B and C to inhibit ovum growth while definitely preventing pseudopregnant proliferation indicates that they cannot affect the progesterone induced reactions leading to ovum stimulation

5 On the basis of these findings we conclude that a chain of reactions *in vivo* may be affected by (or permit the participation of) oestrogens, but that some, like those leading to ovum growth, are labile to compounds of rather definite molecular structure, whereas others, like the processes involving vaginal stimulation, are entered into by a great variety of compounds

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Molecular interaction in monolayers

III Complex formation in lipoid monolayers

By J H Schulman and E Stenhagen*

(*Communicated by E K Rideal, F R S —Received* 29 *August* 1938)

In Parts I and II (Schulman and Rideal 1937) it was shown that on injection into the substrate beneath a uniform coherent monolayer of substances in minute quantities, one of two distinct types of interaction could be observed In one case the injected molecules penetrated the surface film to form a mixed equimolecular monolayer, in the other the injected molecules were adsorbed on to the film to form a double layer, in some cases very rigid or tanned skins being formed by cross-linking It appeared that in the former case equimolecular complexes were formed between the associating molecules, the term "complex" being used to designate a combination of measurable stability in stoichiometric ratio between the two reactants It was further shown that the stability of these complexes in monolayers was due not only to polar interaction between the head groups but also to the operation of the dispersive or Van der Waals forces between the non-polar portions of the respective molecules This latter factor not only imparts a high degree of specificity to the interaction but is in many cases the determinative one which governs their existence in detectable amounts only in the state of orientated monolayers Recently, similar systems in multilayers have been made confirming the importance of the factor of molecular adlineation Small changes in the associating forces, such as in the Van der Waals adhesion or the polar heads or the salt concentration in the substrate, could radically alter the nature and stability of the complexes

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In this work further experimental support is given to these concepts, especially in relation to the association between saturated and unsaturated hydrocarbon chains and aromatic ring structures The previous experiments were carried out with the films at constant area and at definite pressures of 5 or 10 dynes/cm Under these conditions mixed monolayers containing the two reactants only in the ratio of 1 1 were obtained on "penetration" (One molecule in the monolayer to one from the substrate) By experiment over a wide range of areas as well as pressures a number of general types of complex were found to emerge from the investigation Thus it is found that when both reactants are ring structures only 1 1 complexes are stable When a ring structure monolayer is penetrated by a chain reactant, the formation of surface complexes containing one, two or three chain molecules per ring molecule in the monolayer has been established In the inverse case, two chain molecules in the monolayer can link up with one ring structure molecule from the substrate If both reactants are chain molecules, a maximum of three molecules from the substrate can form a solid complex with one molecule in the monolayer In these systems, whilst 1 3 and 1 1 complexes are stable, no evidence for stable 1 2 complexes could be obtained It is found that a monolayer of a *trans* unsaturated chain compound is readily penetrated by a saturated chain compound producing a very stable solid 1 1 complex at high surface pressures Here, evidence for a solid 1 2 complex was obtained, but no 1 3 complex is obtained as occurs with saturated chains Since these stable complexes give solid films, two-dimensional crystalline lattices with different symmetries can be constructed Penetration of monolayers of *cis* unsaturated chain compounds is feeble and the resulting complex is readily decomposed on surface compression, the penetrating molecules being ejected from the surface layer back into the substrate Complexes of these different ratios are obtained when the penetrating agent is in sufficient concentration to enable the 1 1 complex to collapse as a unit When a strong association exists between the reactants as is, for example, found in the case of the penetration of a film of cholesterol by sodium cetyl sulphate injected into the substrate, the quantity of the latter necessary is remarkably small, being only of the order required to form a monolayer on the surface, thus 0 1 mg /100 c c injected beneath a film of cholesterol extended so as to cover 60 A^2/molecule immediately raises the pressure by some 30–40 dynes/cm , although the cholesterol alone only commences to form a coherent film when compressed to areas of *ca* 40 A^2 Under these conditions the two components alone do not measurably affect the surface tension

EXPERIMENTAL

For measuring the change and rate of change of area at constant pressure, produced by the penetration of a substance injected into the substrate beneath a monolayer, two different devices were employed In the first a trough was divided by means of a waxed barrier guided by hooks between two parallel wires above the trough enabling the barrier to move the distance of the trough and at right angles to the sides Leakage between the ends of the barrier and the sides of the trough was prevented by the attachment of long vaselined silk threads both to the barrier and to the sides of the trough On one side of the barrier the monolayer to be investigated was deposited in the usual way Its pressure was controlled by placing a lens of a spreading substance on the surface of the substrate on the other side of the barrier The pressure exerted by the monolayer spreading from the lens (Cary and Rideal 1925) is constant and thus serves as a piston (Langmuir 1935) The following substances were employed as piston fluids with their respective pressures on substrates of *p*H 7 2 in dynes/cm tricresyl phosphate (9 8), castor oil (16 3), triolein (21), and oleic acid (35) This method is restricted to those piston fluids that do not suffer a large change in pressure on injection of the reactant into the substrate

In the second method the monolayer under examination was confined in a Langmuir trough between barrier and glass slide in the usual manner, the movement of the glass slide, however, was automatic, being performed by a reversible motor which was set in motion through relay systems activated by any movement of the barrier suspended from the torsion wire A variation in pressure (either positive or negative) was sufficient to set the slide in motion when the electric contacts on each side of the barrier operated electric relays, a sensitivity of 0 5 dyne/cm being achieved The pressure behind the barrier was either controlled by placing a lens of a piston oil on the surface, or the surface tension of the solution after injection was directly determined by means of the ring method The monolayer could thus be placed on the surface of the substrate, the pressure adjusted to any desired value, and on injection of the reactant the area during the penetration process automatically adjusted itself in equilibrium with the pressure on the torsion head and the rate of penetration could be measured in the usual way, directly or by graphical recording When, as is frequently the case in strongly interacting systems, the monolayer of the complex formed on penetration is a solid, the change of area when the pressure is increased and molecules of the reactant are ejected from the film proceeds so slowly that the automatic device is practically essential for uniform reaction A series of measurements was carried out at different concentrations of the penetrating substance

Molecules containing ring systems penetrating monolayers containing ring structures

In fig 1 is shown the force area characteristic curve of a monolayer of cholesterol spread upon a phosphate buffer of pH 8 at 20° C It forms a liquid condensed film of limiting area of 40 A^2 This is compared with the same film after 4 mg of digitonin have been injected into the substrate of volume 300 c c It is seen that the limiting area of the film has increased to 80 A^2 and the solid film collapses at some 60 dynes/cm well above the collapse points of cholesterol (40 dynes/cm) or of digitonin monolayers (20 dynes/cm) Evidently a very stable 1 1 complex is formed and the digitonin molecule has the same area as the cholesterol molecule at high compressions At pressures less than 25 dynes/cm digitonin in excess of the 1 1 ratio can penetrate the monolayer which is still solid, but this excess is readily ejected on raising the pressure

If with the constant-pressure device a cholesterol film be compressed by an oleic acid piston the waxed barrier will move outwards on injection of the digitonin to an extent exactly equal to the area of the original film of cholesterol as seen in fig 1 at 35 dynes/cm pressure The movement of the barrier is quite rapid and the cholesterol film solidifies in the process

The oleic acid or triolein films used as piston films are unaffected by the digitonin and remain liquid This specificity (shown by digitonin in its action on different monolayers) was noted also in Part I, where the esters cholesterol acetate, lecithin and sphyngosin were found to be unaffected by digitonin or saponin Digitalis does not penetrate and is not adsorbed by cholesterol monolayers, and no complex has been detected by other methods Glycocholic, desoxycholic, cholanic, cholalic, dihydrocholic, taurocholic acids and scymnol form very weak 1 1 complexes with cholesterol monolayers, the mixed films on penetration collapsing at the spreading pressure of the injected substances We shall have occasion to refer to these values in Part IV in relation to their haemolytic activities

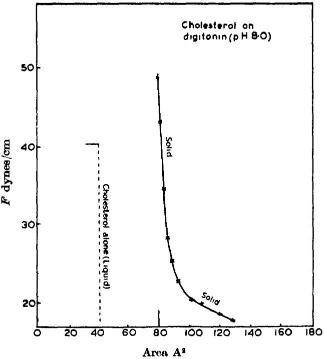

Fig 1

Molecules containing long chains penetrating monolayers containing ring structures

In fig 2 are shown the force area characteristics of cholesterol spread upon a substrate (pH 8) containing saturated sodium cetyl sulphate

It is seen that when surface peptization of the cholesterol film occurs the resulting mixed monolayer is fluid and the limiting area of 59 A² indicates that only a 1 1 complex is stable, the areas of cholesterol and the sodium cetyl sulphate under high compressions being 40 and 19 0 A² respectively As will be seen later the extension in area at lower pressures may proceed smoothly or with a series of breaks in the curve It is difficult to ensure the absence of all hysteresis effects, but the extension of the area in this case suggests that it is due to a solution of sodium cetyl sulphate in the liquid 1 1 complex monolayer When the underlying solution is made dilute, the extension in area beyond 80 A² takes place at constant pressure, being due to the lowering of the surface tension of the water by that concentration of sodium cetyl sulphate

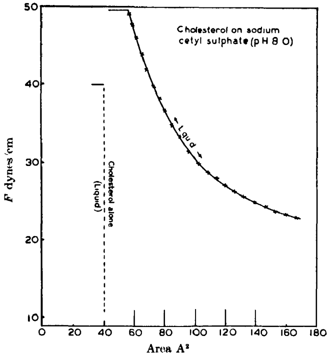

FIG 2

The surface pressures at 60 A²/cholesterol molecule of the 1 1 complexes are dependent on the concentration in bulk of the sodium cetyl sulphate as shown in fig 3 The values depicted in fig 3 are equilibrium pressures (such determination taking several hours), at 20° C, obtained by injecting minute concentrations of sodium cetyl sulphate (0 1 mg) into 300 c c of M/250 phosphate buffer at pH 7 2 on which a cholesterol film has been spread at 60 A²/molecule (area of trough 374 cm ²) Cholesterol films do not register a pressure until they have been compressed to 40 A²/molecule The pressure was obtained by the chainomatic ring method It is instructive to note that such minute concentrations under these conditions can alter the surface tension of the aqueous solution to such large extents The surface pressures registered for a cholesterol film at 60 A² collapse area of the mixed film on a solution of sodium cetyl sulphate reach a maximum at about 0 5 mg /300 c c concentration of about 49 dynes/cm We may note that a saturated sodium cetyl sulphate solution alone only lowers the surface tension 22 dynes/cm at 20° C If the cholesterol film be compressed to 40 A² and the sodium cetyl sulphate be then injected, the equilibrium pressure is about 60 dynes/cm It seems probable that the increase in stability of the film containing cholesterol originally compressed is to be attributed to an adsorbed double layer of sodium cetyl sulphate attached to those cholesterol molecules which have no adjacent sodium cetyl sulphate molecule in the monolayer We can visualize the structure as a partially penetrated cholesterol film with a partially adsorbed double layer of cetyl sulphate molecules as the equilibrium case Thus the packing of the cholesterol molecules in the two cases being different, different equilibrium pressures will be registered It would appear that the complex must be in dissociative equilibrium with sodium cetyl sulphate in the monolayer and this in turn in equilibrium with that substance in the bulk phase Taking the molecular

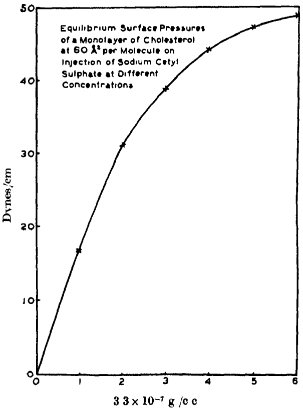

FIG 3

area of the sulphate as 20 A^2, at $F = 16$ and 49 dynes/cm , the difference in the free surface energies involved in the formation of the complex at these two concentrations is $\Delta F = 940$ cal /g -mol The difference of 940 cal /g -mol should, by Gibbs' Law, if true equilibrium be established, be equal to $RT \log C_1/C_2$, where C_1 and C_2 are the equilibrium concentrations of the dissociating cetyl sodium sulphate This is equivalent to about a fivefold variation in concentration, which is in fact approximately equal to the experimental values of 0 10 and 0 50 mg /300 c c , supporting the assumption that the values obtained are true equilibrium values, and that the complex can be described as behaving like a chemical compound

It is interesting to note that if a mixed film of cholesterol and the long-chain cetyl salt be spread upon an aqueous solution with no cetyl sulphate in it, at pH 8 only a very weak complex of 1 1 could be obtained which readily collapsed at 12 dynes/cm (showing the same point as in fig 3 for 300 c c and 0 067 mg in the solution), and from which all the sodium cetyl sulphate could be ejected at pressures as low as 20 dynes/cm leaving a pure cholesterol monolayer If the long-chain octadecyl salt be employed instead of the cetyl, a much greater stability of the mixed film is observed, but in this case a break in the curve suggests that a 1 2 complex is formed at 10 dynes/cm pressure and the 1 1 complex commences to collapse at 30 dynes/cm pressure, the salt being completely ejected only at 50 dynes/cm pressure The increase of two CH_2 groups in the chain has caused a very marked increase in the stability of the complex far greater than the decrease observed in the surface tension lowering of the two sulphates alone which was found to be 8 and 10 dynes/cm respectively

The ejection pressures appear to be reasonably reproducible in these systems where one species is ejected and thus differs from the ordinary collapse phenomenon Several other substances were found to penetrate and form complexes with cholesterol, thus psychosin (0 5 mg /100 c c) gives a very stable complex of 1 2 composition at 55 dynes/cm and a 1 1 complex stable at 65 dynes/cm on a phosphate buffer at pH 8 Somewhat larger concentrations, viz 1 mg /100 c c of stearyl-cholin gives a stable 1 2 complex monolayer stable at 53 dynes/cm This monolayer is interesting in that it exhibits gel-like characteristics similar to the proteins which may be ascribed to the action of the positive quaternary nitrogen associating with the ketonic polar group, thus forming a network on the surface

With stearyl-dimethyl-amino-ethanol (1 mg /100 c c) less stable mixed films are obtained, the 1 2 complex collapsing at 40 dynes/cm and the 1 1 at 50 dynes/cm In this case no gelation could be observed reflecting the weaker association of the secondary substituted nitrogen We shall have occasion to refer to these differences in relation to their haemolytic activities in Part IV

Molecules containing hydrocarbon chains penetrating long-chain structure monolayers

In fig 4 is shown a typical penetration of a hydrocarbon chain system, cetyl alcohol, by a hydrocarbon chain containing reactant (viz sodium cetyl sulphate) on a phosphate buffer at *p*H 8 at 20° C It will be observed

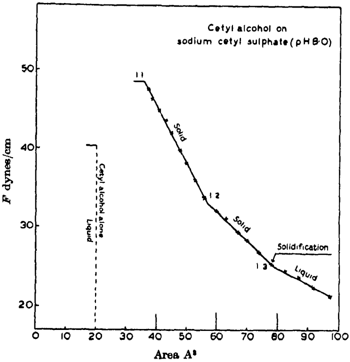

FIG 4

(fig 4) that the film becomes rigid at 78 A^2, presenting a small kink at 59 A^2 and finally collapses at *ca* 38 A^2, corresponding to complexes of composition 1 3, 1 2 and 1 1, of which the 1 2 appears to be relatively unstable The curve was obtained with 2 min intervals between each point If the automatic apparatus be employed and the pressure adjusted to 23 dynes/cm (with a concentration of 1 mg /300 c c of cetyl sulphate) which is just above the collapse pressure of the 1 3 complex, there is a rapid movement of the slide until an area of 78 A^2 is obtained where the film becomes suddenly solid, the change in speed of compression is most marked at this point and then the contraction proceeds along (ii) fig 5, until it comes to rest at a film area of 39 A^2 or the 1 1 complex

In fig 5 is plotted the rate of compression over the area range of 78–39 A^2 in logarithmic form (i) The ejection process follows a unimolecular

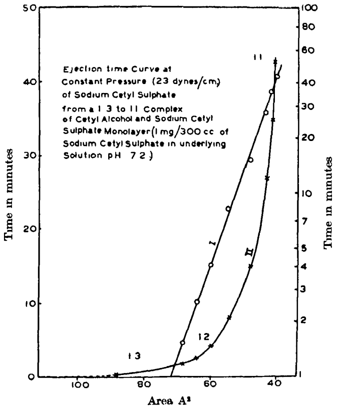

FIG 5

law similar to that expected in the decomposition of a chemical compound in monolayer form, and no departure is observed at an area corresponding to the 1 2 complex

Thus with interpenetrating saturated chain complexes we find that both 1 1 and 1 3 complexes are stable and 1 2 complexes are either not found or possess but a transitory existence The electron diffraction pictures of Havinga and de Wael (1937) and also of Germer and Storks (1938) have shown that in the case of monolayers of barium stearate deposited on a supporting base, the arrangement is that of hexagonal symmetry and not rhombic as in the crystal If the monolayers are regarded as pseudo-crystalline, the distance between the chains is similar to that in the molten material or expanded crystal, and thus free rotation for vertically orientated molecules as shown by Malkin (1933) occurs The close packing in monolayers of simple molecules undergoing free rotation involving a hexagonal pattern can be described as conforming to type C_3^I by A L Patterson (1935) Rhombic packing for two species in the ratios 1 1 and 1 3 can readily be constructed of the C_{2v}^I or C_{2v}^{IV} types, but the 1 2 ratio C_{2v}^I involves a looser packing, hence it might be supposed that the 1 2 form would only be transitory as the experiments suggest and that the structure of the cell lattice of the mixed monolayer is really rhombic as in the fatty acids Further evidence for the formation of monolayer lattices with different symmetries is given by an examination of the complexes formed by *cis* and *trans* unsaturated oleyl and elaidyl alcohols by penetration of sodium cetyl sulphate

Effect of cis *and* trans *isomerism on the stability of complexes between long-chain hydrocarbon compounds*

It will be noted in fig 6 that *trans*-elaidyl alcohol is readily penetrated by the cetyl sodium sulphate (3 mg /100 c c) to form a solid film at *ca* 60 A^2 corresponding to a 1 2 complex, stable at 30 dynes/cm , the 1 1 complex (40 A^2) collapsing as a unit at very high pressures, *ca* 50 dynes/cm This is in marked contrast to the behaviour of the oleyl alcohol (already noted in Part I), in which only a liquid 1 1 complex is noticed at 35 dynes/cm , which complex decomposes on elevation of the pressure by ejection of the cetyl sodium sulphate This compressed oleyl alcohol film on the cetyl sulphate solution collapses at *ca* 22 A^2, and at a pressure of some 48 dynes/cm , being compared to the force area curves of the alcohol alone collapsing at 35 dynes/cm showing the increased stability due to the double layer film of adsorbed cetyl sulphate Evidence for the interlocking of saturated chains was presented by Lyons and Rideal (1929), and of *trans* unsaturated with saturated chains by Marsden and Rideal (1938), and it is interesting to note that the solid 1 2 and solid 1 1 complexes are formed in this case, but that the 1 3 is absent suggesting that the interlocking, when a double bond in the *trans* position is present in one species, is such that free rotation is either hindered or prevented and that these mixed films of saturated and unsaturated substances probably do not possess rhombic symmetry A similar apparent hindering of free rotation and thus elimination of rhombic symmetry and the formation of 2 1 complexes can be effected by the introduction of a non-symmetrical molecule instead of a chain

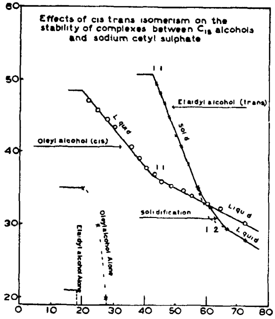
Effects of cis trans isomerism on the stability of complexes between C_{18} alcohols and sodium cetyl sulphate
1 1
Elaidyl alcohol (trans)
Solid
Liquid
Oleyl alcohol (cis)
1 1
Liquid
Solidification
1 2
Liquid
Elaidyl alcohol Alone
Oleyl alcohol Alone
F dynes/cm
Area A²
60
50
40
30
20
0
10
20
30
40
50
60
70
80

FIG 6

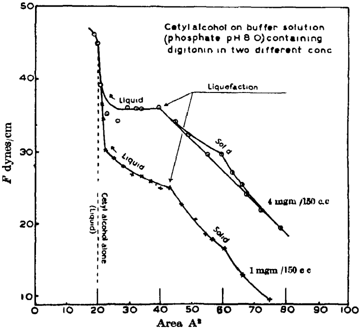
Cetyl alcohol on buffer solution (phosphate pH 8 0) containing digitonin in two different conc
Liquefaction
Liquid
Solid
Liquid
Solid
4 mgm /150 c.c
1 mgm /150 c c
Cetyl alcohol alone (Liquid)
F dynes/cm
Area A²
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100

FIG 7

Molecules containing ring systems penetrating long-chain structure mixed layers

In Part I it was observed that digitonin could penetrate films of cetyl alcohol but that saponin could not The penetration is not as strong as into a film of cholesterol In fig 7 are shown the curves for the effects of digitonin in two different concentrations on monolayers of cetyl alcohol It will be seen that there is strong evidence for a 2 1 complex, for at 40 A^2 the film suddenly liquefies and presumably the digitonin is ejected, leaving at 20 A^2 a film exhibiting all the characteristics of cetyl alcohol Digitonin, as has been shown, with films mixed with cholesterol, has an area of 40 A^2, hence the mixed film of apparent area of 40 A^2 must contain two alcohol molecules to one of digitonin At 60 A^2 there is a possible break in the curve suggestive of the presence of an unstable 1 1 complex As in the case with cholesterol sodium cetyl sulphate complexes, the stability pressures are dependent on the bulk concentration of digitonin

We are grateful to Professor G Blix, Uppsala, for permitting samples to be used of the original Hammarsten preparations of bile acids, to Dr R Mathieu, Paris, for samples of stearyl-cholin and stearyl-dimethyl-amino-ethanol, and to the I C I , Blackley, for a sample of psychosin chloride and sodium cetyl sulphate

Our thanks are due to Professor E K Rideal for valuable help and encouragement, to Dr W A Wooster for assistance in construction of two-dimensional crystal lattices, and to the Medical Research Council for a personal grant to one of us (J H S)

SUMMARY

Two new methods are described for investigating the process of penetration of monolayers by substances injected into the substrate In one the monolayer is confined by a movable barrier and maintained at constant pressure by means of a non-reactive piston fluid, the area change on injection being noted In the other an electrically operated Langmuir trough is employed, the slide retaining the monolayer travelling automatically so as to retain the monolayer at constant pressure during the process of penetration or ejection of one constituent

It is found that molecules containing ring systems such as digitonin, on penetrating a ring structure monolayer such as cholesterol form only 1 1 complexes, a number of cases of specific penetration in these systems are

examined A saturated chain molecule such as sodium cetyl sulphate penetrating a ring structure monolayer such as cholesterol, forms a 1 1 complex The surface pressure of the complex is found to be dependent on the concentration of the reactant in the substrate and to be in equilibrium with it The difference in surface-free energies of formation of the complex with different bulk concentrations of reactant can be evaluated both from the surface-pressure differences as well as from the variations in the bulk concentrations thus showing an experimental justification of Gibb's Law and that the complexes behave as chemical compounds Other examples of this type were found to form complexes of composition both 1 2 and 1 1 Saturated chain reactants, e g sodium cetyl sulphate on penetrating saturated chain monolayers, e g cetyl alcohol, form stable complexes of composition 1 3 and 1 1, whilst an unstable 1 2 complex can be identified under certain conditions The rate of decomposition of a complex monolayer at constant pressure follows the unimolecular law

The presence of 1 3 and 1 1 complexes giving solid mixed monolayers with saturated chains and of 1 2 and 1 1 complexes with *trans* unsaturated chains suggests that the monolayer lattices of these complexes correspond to different "crystal" symmetries or types, and if this proves to be the case on crystallographic examination, Patterson's classification appears to be applicable

Marked differences, due to *cis* and *trans* isomerism, are obtained on penetration, supporting the concept of interlocking chains

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612 86 595 792 *Nemeritis*

Further experiments on olfactory conditioning in a parasitic insect The nature of the conditioning process

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(*Communicated by A D Imms, F R S —Received* 14 *September* 1938)

Introduction

In the course of a recent paper (Thorpe and Jones 1937) it was shown by means of experiments with an olfactometer of the McIndoo type, that the parasitic ichneumonid, *Nemeritis canescens* (Grav), is strongly attracted by the odour of the larva of its normal host, the Mediterranean flour moth, *Ephestia kuhniella* (Zell) This response is an inherent one, and is manifested throughout the life of the adult parasite The insect can also be reared on the larvae of the closely allied genera of wax moths, *Meliphora* and *Galleria* Whereas the larvae of *Meliphora* exert no attraction whatever on parasites reared from *Ephestia*, they exert a quite definite attraction on individuals which have been reared from *Meliphora* or which have been exposed to contact with *Meliphora* larvae for a period shortly after emergence from the cocoon Insects which have been treated in this way, when given the choice in the olfactometer between *Meliphora* and *Ephestia*, whilst still preferring the latter, show significant attraction to the former, such that the proportion choosing *Ephestia* is reduced from 85 to 65 8% It was concluded from these experiments that *Nemeritis* has an oviposition response to *Ephestia* larvae, but that an additional oviposition response to the odour of *Meliphora*, a response which is entirely lacking in the normal animal, can be conditioned by contact with the new host Such conditioning can take place either in the pre-imaginal stage when the larva is feeding inside its host caterpillar, or in the adult stage immediately after emergence It was shown that even though individuals reared on the abnormal host were isolated from all contact with that host immediately on emergence, they still retained the ability to respond to the odour of the abnormal host in which they had been reared It was concluded that all these results could be interpreted as the establishment of a simple conditioned response to a new odour, and that conclusion has been confirmed by the subsequent work which is described in the present paper

Many points of interest needing further investigation arise out of the experiments summarized above The first of these is the question of the real nature of the conditioning, and to answer this has been the chief object of the investigation here described Besides this, an attempt has been made to determine more accurately the duration of the conditioning effect, the effect of variation in period of exposure to the conditioning stimulus, and the facility with which the conditioning of the new stimulus can be established at different ages Deconditioning, or the experimental extinction of the conditioning effect, has also been further studied, as well as the question of the insect's response to its own odour and the bearing of this on the problem of superparasitism

METHODS

The methods employed have been essentially the same as those described in the previous paper, although various modifications have been introduced to ensure greater accuracy The chief of these has been the equipment of the olfactometer with a pair of flowmeters of a simple standard type One flowmeter is attached to the inlet tube of each arm of the olfactometer and the two are calibrated so that it is possible to ensure that an equal volume of air is flowing down each tube The level was usually adjusted to give a flow of approximately 500 c c /min The meter itself is illustrated in fig 1, which is self-explanatory As before the temperature and humidity were controlled at 25° C and 65-70 % relative humidity Although in previous experiments the air had been drawn through the apparatus by means of a suction pump, it was thought that better results might be obtained, and conditions might be more easily controlled, if instead of suction the air was blown through the apparatus from the compressed air supply The apparatus was accordingly fitted up to allow of this, but although a considerable number of experiments were performed with it, it was found eventually that the efficiency was not as great as with the suction type of instrument, the use of which was therefore resumed The fact that the pressure instrument was less satisfactory was shown by control tests with normal insects given the choice *Ephestia* against "Blank" when the proportion choosing the *Ephestia* dropped significantly The reason for this is difficult to determine, but is probably concerned with the greater quantity of rubber tubing which is necessitated when compressed air is used Although the rubber tubing employed was treated with hot acid and alkali and boiled to remove sulphur, it is impossible to get rid of all odour This odour is probably a source of error, masking to some extent the odour of the host or substance which is being used in the experiments Certain special methods which were adopted for particular phases of the work are described below under the appropriate headings As in the previous work the position of the attractant was alternated from one arm of the apparatus to the other at each experiment, so that in one half of each set of experiments the main stimulus would be in the right limb and in the other half in the left In this way errors due to any unavoidable bias inherent in the instrument are eliminated

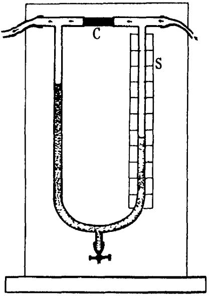

FIG 1 Diagram of flowmeter Arrows indicate direction of air flow C = capillary tubing fused into flow tube S = millimetre scale

I am indebted to my laboratory assistant Mr F Bloy for his valuable help in designing and working apparatus and in maintaining stocks during the course of these experiments

NATURE OF CONDITIONING

As stated above, the chief problem raised by the earlier experiments is that of the actual nature of the conditioning which is obtained In Pavlov's famous experiments the stimulus A, which is to be conditioned, and which originally does not cause any response, is offered simultaneously with a second unconditioned stimulus B, which causes a distinct and specific response, the stimuli being reinforced, to use Pavlov's expression, by the presentation of food When this process has been repeated several times an association is formed between the two stimuli, with the result that stimulus A will now alone produce the response, and a conditioned reflex is said to have been established That the state of affairs in *Nemeritis* is not so simple is evinced by the fact that modification of the conditions of the larval life appear capable of influencing the oviposition response of the adult Now, in this case, there is clearly no association between two simultaneously presented stimuli since, of course, the insect does not become capable of laying eggs until after it has emerged from its host It appears therefore, that it is not a question of the direct association of two stimuli, but rather a case of what seems to be a general attraction exerted by an odour which, in an earlier stage of existence, had been characteristic of the environment In our earlier experiments, this conditioning had been brought about, not only in the pre-imaginal stage, but also in the adult, as the result of close contact with the abnormal host on emergence from the pupa In this case, however the possibility of an association being developed between two simultaneously presented stimuli, (1) the unconditioned stimulus of the presence of a host caterpillar for oviposition and (2) the odour of the new host, could not be ruled out since the conditioning had been brought about by the simple method of placing the newly emerged parasites for a week or more in a jar containing crumbled honeycomb densely infested with larvae and pupae of *Meliphora* As explained in the Introduction of the previous paper, although normal *Nemeritis* are not significantly attracted by the odour of *Meliphora* other factors such as the texture of the cocoon, the movement and size of the host and so forth, come into play in eliciting the stabbing reflex and the actual laying of the eggs, and, if normal *Nemeritis* females are confined closely with *Meliphora* larvae, a certain amount of stabbing will take place and a small number of eggs will be laid Consequently, in these experiments, the possibility of an association between the smell of the new host and the act of oviposition had not been ruled out Therefore the first point to investigate was whether the adults could be conditioned merely by being exposed to the *odour* of *Meliphora* larvae without being allowed actual contact with them For this purpose a "conditioner" was constructed which consisted simply of a piece of wide glass tubing containing food and water for the parasites, through which is drawn a stream of air of constant humidity which has

TABLE I RESPONSES OF 200 INDIVIDUALS OF *NEMERITIS CANESCENS* (REARED FROM *EPHESTIA*) TO LARVAE OF *MELIPHORA* IN OLFACTOMETER

(Series 1–9 with pressure olfactometer Series 10–19 with suction olfactometer)

Exp no	No in *Meliphora* arm	No in "Blank" arm	Total
1	20	21	41
2	16	23	39
3	13	11	24
4	15	9	23
5	12	14	27
6	10	10	20
7	9	13	22
8	10	9	19
9	12	10	22
10	16	9	25
11	12	12	24
12	13	11	24
13	10	14	24
14	14	12	26
15	13	10	23
16	10	12	22
17	13	9	22
18	13	12	25
19	10	13	23
Total	241 = 50 8 %	234 = 49 2 %	475
Data from Table IV of previous paper suction instrument	184 = 50 8 %	178 = 49 2 %	362
Total	425 = 50 8 %	412 = 49 2 %	837

Standard error = $\sqrt{pq/n} = \pm 1\ 73\ \%$

previously passed through a chamber densely packed with *Meliphora* larvae In this way the insects are exposed to the smell of *Meliphora* larvae without having opportunity of actual contact with them The results of these experiments are summarized in Tables II and III It will be seen that insects treated in this manner for a week or more, and then offered the choice of *Meliphora*—"Blank" in the olfactometer, choose the *Meliphora*-containing arm a significantly large number of times, namely 59 % This figure is very close to that given in Table VIII in the previous paper, and in fact is not significantly different from it From this it appears that the "conditioner" method of establishing the response is as effective as actual contact and, therefore, there is no reason to suppose that the occasional stabbing and oviposition which take place when the insects are in contact with the caterpillars play any part in the conditioning process It is seen, therefore, that the pre-imaginal conditioning and the imaginal conditioning can be regarded as being of precisely the same nature

TABLE II *NEMERITIS* EXPOSED TO THE ODOUR OF *MELIPHORA* LARVAE FOR 1 WEEK AND TESTED IMMEDIATELY IN OLFACTOMETER, *MELIPHORA* AGAINST ' BLANK"

Series No	No in *Meliphora* arm	No in ' Blank" arm	Total	No of exps in series	No insects used
1	113 = 56 4%	88	201	10	45
2	74 = 63 2	43	117	4	(45)
3	86 = 59 0	60	146	8	35
4	85 = 50 5	83	168	8	24
5	101 = 55 5	81	182	10	20
6	128 = 64 3	71	199	9	30
7	138 = 59 3	95	233	10	35
8	86 = 70	37	123	6	30
Total for series 1 6 (pressure olfactometer)					
	587 = 58%	426	1013	49	154
		S E = ± 1 58%			
Total for series 7 and 8 (suction olfactometer)					
	224 = 63%	132	356	16	65
		S E = ± 2 65%			
Total for series 1 8					
	811 = 59%	558	1369	65	219
		S E = ± 1 39%			

$$\chi^2 = \frac{\Sigma(ap) - np}{\bar{p}q} = \mathbf{15\ 76} \quad \text{When } P = 0\ 05,\ \chi^2 \text{ is } \mathbf{14\ 067}$$

Since it might be argued that the establishment of this conditioned response was dependent upon some slight similarity in odour between *Ephestia* and *Meliphora* which are as pointed out above, closely related insects, it was thought advisable to attempt to condition the parasites to some entirely strange odour which could hardly have any significance for them For this purpose it seemed desirable to find some substance which normally exerted neither attraction nor repulsion, but unfortunately it has not yet been possible to find such a substance Preliminary experiments were carried out employing a number of different substances including ammonia, valerianic acid, butyric acid, clove oil, cedar wood oil, camphor, allyl alcohol, isopropyl alcohol, and butyl alcohol The use of several of these proved impracticable owing to their high rate of evaporation, and of those which could be conveniently employed none appeared completely neutral Camphor was easy to work with, but gave very different results with slight variations in concentration Under some circumstances it was strongly repellent, under others slightly attractive, and for these reasons its use was not continued Accordingly, for the time being the attempt to find a neutral odour was abandoned and experiments were started to see if it was possible to condition *Nemeritis* so that they are attracted by a non-toxic odour normally repellent to them

TABLE III COMPARISON OF BEHAVIOUR OF UNCONDITIONED *NEMERITIS* WITH OTHERS EXPOSED TO ODOUR OF *MELIPHORA* FOR A PERIOD IMMEDIATELY AFTER EMERGENCE FROM THE PUPA BOTH LOTS REARED FROM *EPHESTIA*

	Meliphora	"Blank"	
	a	*b*	Total
Unconditioned *Nemeritis**	425 = 50 8%	412 = 49 2%	837
	c	*d*	
Nemeritis exposed to odour of *Meliphora*	811 = 59%	558 = 41%	1369
Total	1236	970	2206

$$\chi^2 = \frac{(ad - bc)^2 N}{(a+b)(c+d)(a+c)(b+d)} = 15\ 1 \qquad P < 0\ 01$$

* The first set of figures includes results published in Table IV of previous paper

For this purpose cedar wood oil was found to be fairly satisfactory † It is true that it is toxic in high concentrations, but provided the dose is not too great, and provided the insects are not allowed contact with the oil they will survive long treatments without suffering any apparent harm ‡ Of course, as all investigators with olfactory stimuli have found, there is very great difficulty in standardizing the odour It is difficult, if

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† It might be objected that the use of such a substance as cedar wood oil will not give information concerning the olfactory responses, since essential oils in general cause repulsion, not only by means of the true olfactory organs but likewise by their irritating effect upon organs normally receptive of other types of stimuli Such organs are situated on regions of the body other than the antennae and perhaps also upon the respiratory tract (Hartwell 1924 Valentine 1931) This objection however, does not seem to apply in the case of *Nemeritis* since insects in which the antennae have been amputated while able both to feed and fly and otherwise behave in an apparently normal manner are no longer repulsed by the odour of cedar wood oil but are as indifferent to it as they are to the odour of their host (see Thorpe and Jones 1937)

‡ For instance, prolonged treatment with the vapour of cedar wood oil does not in the least diminish the ability of *Nemeritis* to respond to the odour of *Ephestia*

TABLE IV *NEMERITIS* EXPOSED TO ODOUR OF *MELIPHORA* LARVAE FOR 1 WEEK, ISOLATED FOR 2 DAYS AND TESTED IN OLFACTOMETER, *MELIPHORA* AGAINST "BLANK"

Series no	No. in *Meliphora* arm	No. in "Blank" arm	Total	No. of exps in series	No. insects used
1	99 = 46 %	116	215	11	30
2	40 = 56	31	71	6	(30)
3	29 = 42	40	69	3	30
4	37 = 76	11	48	4	15
5	73 = 52	66	139	6	30
6	112 = 59	78	190	9	55
Total for series 1–3 (pressure olfactometer)					
	168 = 47.4 %	187	355	20	60
		S.E. = ± 2.65 %			
Total for series 4–6 (suction olfactometer)					
	222 = 59 %	155	377	19	100
		S.E. = ± 2.57 %			
Total for series 1–6					
	390 = 53.3 %	342	732	39	160
		S.E. = ± 1.85 %			

For series 1–6 $\chi^2 = \frac{\Sigma(ap) - np}{pq} = \mathbf{21.70}$ When $P = 0.05$, $\chi^2 = \mathbf{11.070}$

For series 4–6 $\chi^2 = \mathbf{8.932}$ When $P = 0.05$, $\chi^2 = \mathbf{5.991}$

TABLE V *NEMERITIS* EXPOSED TO THE ODOUR OF *MELIPHORA* LARVAE FOR 1 WEEK, THEN ISOLATED FOR 4 DAYS AND TESTED IN OLFACTOMETER, *MELIPHORA* AGAINST "BLANK"

Series no	No. in *Meliphora* arm	No. in "Blank" arm	Total	No. of exps in series	No. insects used
1	29 = 42 %	40	69	4	20
2	48 = 53	43	91	5	20
3	67 = 58	47	114	8	55
Total for series 2 and 3 (suction olfactometer)					
	115 = 56 %	90	205	13	75
		S.E. = ± 3.49 %			
Total for series 1–3					
	144 = 52.5 %	130	274	17	95
		S.E. = ± 3.02 %			

$\chi^2 = \frac{\Sigma(ap) - np}{pq} = \mathbf{4.834}$ When $P = 0.05$, $\chi^2 = \mathbf{5.991}$

TABLE VI *NEMERITIS* EXPOSED TO THE ODOUR OF *MELIPHORA* LARVAE FOR 1 WEEK, ISOLATED FOR 6 DAYS AND TESTED IN OLFACTOMETER, *MELIPHORA* AGAINST "BLANK"

Series no	No in *Meliphora* arm	No in "Blank" arm	Total	No of exps in series	No of insects used
1	44 = 50 %	44	88	4	26
2	44 = 66	23	67	5	20
3	46 = 50 5	45	91	6	25
4	49 = 68	23	72	5	15
5	22 = 56	17	39	4	12
	205 = 57 4 %	152	357	24	98

S E = ± 2 65 %

Total with series 2 and 4 revised (see text p 379)

	163 = 53 7 %	140	303	20	98

S E = ± 2 87 %

$$\chi^2 = \frac{\Sigma(ap) - np}{pq} = 2\ 327 \quad \text{When } P = 0\ 05,\ \chi^2 = 9\ 488$$

not impossible, to ensure that the odoriferous substance is always present in a given sample of air in the same proportion and even if one succeeds in doing this, there are difficulties due to the adsorption of the substance on the walls of the apparatus There is consequently always bound to be a large error associated with experiments of this nature For conditioning the cedar wood oil it was found more satisfactory to place the animals in a desiccator, the lower compartment of which contained a muslin pad soaked in the oil The insects were confined in the upper compartment which was separated from the lower by a gauze screen, and were supplied with food and water For use in the olfactometer a piece of No 1 Whatman filter paper, 2 sq cm in area, was saturated with cedar wood oil and placed in contact with the wall of the bait tube This simple method was found to give fairly satisfactory results Table IX (Exps 1–6) shows the repellent effect exerted on *Nemeritis* by the odour of cedar wood oil It will be seen that although there is some variation, the results are fairly consistent and that the data are compatible with a hypothesis of homogeneity The summary of the figures shows that 76 % of the insects avoided the tube containing the oil In the next series Table IX (Exps 7–18), the results of a total of 1125 tests are given in which 300 insects were employed, which had previously been exposed to the vapour of cedar wood oil for a period of 7 days It will be seen that the proportion avoiding the cedar wood oil is significantly reduced, but that no actual preference for cedar wood oil has been developed Although the figures are in the neighbourhood of 50% the distribution is significantly different from a random one The insects have not become entirely indifferent to the oil and clearly some trace of the repellent effect still remains

DURATION OF CONDITIONING EFFECT

In the previous paper it was shown that the conditioning effect lasted over some days, although its exact duration was not determined It was accordingly of interest to find out how rapidly the extinction of the newly conditioned reflex takes place when the animal is isolated from the odour of the abnormal host Accordingly after the odour of *Meliphora* had been established as the conditioned stimulus by a week in the "conditioner" the insects were isolated and tested in the olfactometer *Meliphora* against "Blank" at intervals of 2 days These results are summarized in Tables IV–VI The first group of results in each of the first two tables comprises experiments with the less efficient type of olfactometer In the second group a suctional olfactometer was employed It will be seen from these that there is a diminution with age in the proportion which seek *Meliphora* The results given in these tables have been summarized in curve form (fig 2) It will be seen that although fluctuation is considerable there is a general tendency for a falling-off in the conditioning effect of which but little trace appears to remain after about the eighth day The slight peak in the curves about the sixth or seventh day is noteworthy and appears to be due to a sort of facilitation The conditioning effect although reduced is not completely extinct and is rapidly re-established when the insects are again exposed to the odour of *Meliphora* as is of course the case during a series of runs through the olfactometer It was found that the higher figures for the sixth day (Table VI, series 2 and 4) were mainly obtained towards the end of a series of experiments If the first three experiments only of each series were taken, the value dropped very considerably as is shown by the curve 2*B* in which the last two of each set of runs are omitted That the curves are not entirely regular is not surprising Pavlov has noted that even with stimuli of exactly constant strength, and with constant environmental conditions, a situation which of course cannot be duplicated in the case of experiments with odour, fluctuations in the course of experimental extinction are often observed These fluctuations are of a rhythmic character and are evidently due to internal factors, and Pavlov expresses the view that once a stimulus has become firmly conditioned it can never be completely extinguished and is always liable to recur It will be noticed also that whereas the figures in Table IX, series 1–6, are all of reasonable homogeneity, those after conditioning to cedar wood oil (series 7–18 and Table X, 1–10) are much more erratic The reason for this is obscure unless it is due to accidental variation in the degree of exposure of different individuals to what is after all a toxic substance It will be seen that there is not this degree of variation in the insects conditioned to *Meliphora* with the exception of those used after 2 days' isolation (Table IV) It is

TABLE VII *NEMERITIS* EXPOSED TO THE ODOUR OF *MELIPHORA* LARVAE FOR 36–48 HR AND TESTED IN OLFACTOMETER *MELIPHORA* AGAINST "BLANK" AFTER VARYING PERIODS OF ISOLATION

Series no	No in *Meliphora* arm	No in "Blank" arm	Total	No of exps in series	No insects used
	Experiments directly after removal from "conditioner"				
1	49 = 60 %	32	81	4	30
2	65 = 59	45	110	4	40
3	45 = 70	19	64	4	25
4	70 = 62	42	112	3	45
Total	229 = 62 5 %	138	367	15	140
		S E = ± 2 61 %			
	Experiment after 24–36 hr isolation				
5	47 = 50 %	47	94	4	30
		S E = ± 5 16 %			
	Experiments after 48 hr isolation				
6	31 = 48 %	33	64	4	23
7	49 = 69	22	71	4	25
8	64 = 60	43	107	4	35
Total	144 = 59 5 %	98	242	12	83
		S E = 3 21 %			
	Experiments after 4 days' isolation				
9	40 = 57 %	30	70	4	24
10	77 = 66	40	117	4	45
Total	117 = 62 5 %	70	187	8	69
		S E = ± 3 66 %			
	Experiments after 6 days' or more isolation				
11	18 = 62 %	11	29	4	10
12	35 = 53	31	66	4	25
13	57 = 56	44	101	4	30
Total	110 = 56 %	86	196	12	65
		S E = ± 4 77 %			

TABLE VIII SUMMARY OF FIGURES FROM TABLES II–VII
SHOWING EXTINCTION OF CONDITIONING EFFECT

(N B Only results of experiments with the suction olfactometer are included in this table)

	No in *Meliphora* arm	No in 'Blank' arm	Total	No of exps	No of insects	S E χ^2	$\frac{\Sigma(ap) - n\bar{p}}{p\bar{q}}$	Value of α^2 for $P = 0\ 05$
A Exps immediately after conditioning period	453 = 62 6%	270 = 37 4%	723	28	160	±1 86%	19 318	19 675
B Exps 48 hr after conditioning period	366 = 59 2	253 = 40 8	619	31	183	±2 01	14 837	11 070
C Exps 4 days after conditioning period	232 = 59 1	160 = 40 9	392	21	144	±2 52	—	—
D Exps 6 days after conditioning period	273 = 54 6	226 = 45 4	499	32	163	±2 24	—	—

Comparing sets A and D $\chi^2 = \frac{(ad - bc)^2 N}{(a+b)(c+d)(a+c)(b+d)} = 7\ 73$ When $p = 0\ 05$ $\chi^2 = 3\ 84$ $P < 0\ 01$

TABLE IX EXPERIMENTS TO DETERMINE THE EFFECT ON THE RESPONSES OF *NEMERITIS* OF EXPOSURE TO THE ODOUR OF CEDAR WOOD OIL

Series no	No in arm containing cedar wood oil	No in 'Blank' arm	Total	No of exps in series	No of insects used
	Control experiment with unconditioned insects				
1	6 = 15%	34	40	3	21
2	23 = 31	50	73	6	35
3	51 = 25	154	205	11	22
4	9 = 18 9	40	49	4	25
5	4 = 16 5	20	24	1	24
6	14 = 16	73	87	4	30
Total	107 = 22 4%	371 = 77 6%	478	29	157

S E = ±2 29% $\chi^2 = \frac{\Sigma(ap) - np}{pq} = 8\ 3750$ When $P = 0\ 05$ $\chi^2 = 11\ 070$

Experiments with insects exposed to odour of cedar wood oil for 7 days and tested immediately in olfactometer

7	50 = 47%	56	106	8	32
8	25 = 35	47	72	4	20
9	12 = 39	19	31	4	11
10	2 = 18	9	11	1	11
11	66 = 34	130	196	17	32
12	74 = 49	77	151	10	33
13	34 = 53	30	64	4	21
14	28 = 36	49	77	6	20
15	38 = 41	54	92	7	20
16	28 = 68	13	41	5	11
17	69 = 55	56	125	4	40
18	83 = 52	76	159	4	48
Total	509 = 45 5%	616 = 54 5%	1125	74	299

S E = ±1 50% $\chi^2 = \frac{\Sigma(ap) - n\bar{p}}{p\bar{q}} = 40\ 15$ When $P = 0\ 05$, $\chi^2 = 19\ 765$

Comparing series 1–6 with series 7–18 $2 = \frac{(ad - bc)^2 N}{(a+b)(c+d)(a+c)(b+d)} = 25\ 05$

When $P = 0\ 05$, $\chi^2 = 3\ 841$ $P < 0\ 01$

curious that the peak of the heterogeneity should occur in both *Meliphora* and cedar wood oil experiments at the second or third days It may perhaps indicate a rhythmic fluctuation in the process of extinction of the conditioning although much more extensive data would be necessary to establish this with certainty

TABLE X *NEMERITIS* EXPOSED TO THE ODOUR OF CEDAR WOOD OIL FOR 7 DAYS AND TESTED IN OLFACTOMETER AFTER VARYING PERIODS OF ISOLATION

Series no	No in arm containing cedar wood oil	No in 'Blank' arm	Total	No of exps in series	No of insects used
	Nemeritis conditioned to cedar wood oil for 7 days and then tested after 1–3 days' isolation				
1	34 = 42 %	47	81	6	20
2	2 = 4	47	49	2	30
3	52 = 66	26	78	4	
4	32 = 36 7	55	87	6	25
5	35 = 47	39	74	4	
6	9 = 35	17	26	4	10
7	49 = 45	59	108	5	30
8	48 = 61	30	78	4	20
9	37 = 41	53	90	4	26
10	66 = 37	111	177	5	40
Total	364 = 43 %	484 = 57 %	848	44	201
	S E = ± 1 72 % $\chi^2 = \frac{\Sigma(ap) - np}{pq} = 64\ 53$ When $P = 0\ 05$ $\chi^2 = 16\ 919$				
	Experiments after 4 days' isolation				
11	28 = 34 %	54	82	7	20
12	36 = 51	34	70	4	23
13	54 = 45	66	120	4	35
14	44 = 41	63	107	4	33
Total	162 = 42 8 %	217 = 57 2 %	379	19	111
	S E = ± 2 57 % $\chi^2 = \frac{\Sigma(ap) - n\bar{p}}{p\bar{q}} = 4\ 999$ When $P = 0\ 05$ $\chi^2 = 7\ 815$				
	Experiments after 6 days isolation				
15	14 = 27 %	37	51	4	15
16	53 = 49	55	108	4	35
17	26 = 36	46	72	4	22
Total	93 = 40 5 %	138 = 59 5 %	231	12	72
	S E = ± 3 29 % $\chi^2 = \frac{\Sigma(ap) - np}{\bar{p}\bar{q}} = 7\ 524$ When $P = 0\ 05$, $\chi^2 = 5\ 991$				
	Experiment after 20 days' isolation				
18	10 = 18 %	46 = 82 %	56	4	18

EFFECT OF VARYING LENGTHS OF EXPOSURE TO THE NEW STIMULUS

It became obvious in the course of the 1936–7 work that considerably shorter exposure than 7 days would result in a conditioning effect Various casual observations had, however, showed that a period of a few hours was not sufficient to produce any effect at all, and consequently a few experiments were carried out to determine what was the actual length of time in the "conditioner" necessary to produce a measurable result Although the number of experiments has not been very great, it seems clear from the results shown in Tables IV–VII that a conditioning period of 36 hr gives approximately the same degree of response to *Meliphora* as does a period of 7 days A few lots of insects conditioned for this short time were then isolated and their responses tested at intervals From this, see Table VII, it appears that there is no significant difference in the rate of extinction of conditioning These results are also summarized in fig 2

Facility of conditioning at different ages

From general considerations one might perhaps expect that freshly emerged insects would be more readily conditioned than older individuals It does not appear however, that the ability to become conditioned to the new host decreases to any great extent with age, provided of course that the insects are still in a healthy and active condition While the figures given in Table XI do show a lower proportion attracted to *Meliphora* when compared with Tables II and VII the difference is hardly significant

TABLE XI EXPERIMENTS TO TEST THE RESPONSES OF FORTY-FIVE INDIVIDUALS OF *NEMERITIS* ISOLATED UNTIL THE AGE OF 14 DAYS AND THEN CONDITIONED TO *MELIPHORA* FOR 48 HR

Exp no	*Meliphora*	"Blank"	Total
1	13	7	20
2	18	12	30
3	10	14	24
4	12	12	24
5	23	11	34
6	21	13	34
7	18	19	37
8	16	11	27
Total	131 = 57 %	99 = 43 %	230
	S E ± 3 29 %		

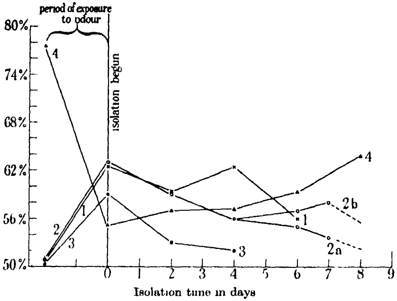

FIG 2 Curves showing rate of extinction of conditioning effect (Diagram summarizing figures given in previous tables)

Conditioning to larvae of *Meliphora*

(1) —x— Average proportion attracted to *Meliphora* before and after a period of 48 hr exposure to odour Results for all experiments with suction olfactometer

(2) —○— Average proportion attracted to *Meliphora* before and after a period of 6 days' exposure to odour Results for all experiments with suction olfactometer (*a*) all results, (*b*) omitting last three runs of series (for explanation see text, p 379)

(3) —●— Curve for results of experiments with both suction and pressure olfactometers

Conditioning to cedar wood oil

(4) --▲-- Average proportion *repelled* by odour of cedar wood oil before and after a period of exposure to the odour

DECONDITIONING

It was shown in the previous work that if insects which have been reared on *Meliphora* are subsequently deconditioned by contact with *Ephestia*, the effect of the pre-imaginal environment, although reduced, is not entirely removed There is in fact a kind of residual effect which still results in a significantly greater proportion of individuals seeking the *Meliphora*, even when offered in competition with *Ephestia* If *Meliphora* only is offered in the olfactometer then deconditioning has no effect in reducing the influence of the larval environment, and the tendency to enter the arm containing the *Meliphora* rather than the blank arm is just as great as if the insect had never been in contact with the *Ephestia*. To test whether conditioning in the adult stage is as stable as conditioning established during the pre-imaginal life a similar experiment was performed, in which, after a week's exposure to *Meliphora* in the conditioner, insects were subjected to the odour of *Ephestia* and then tested *Meliphora* against "Blank". The results of this experiment are shown in Table XII.

TABLE XII. EXPERIMENTS TO SHOW THE RESPONSES OF NINETY INDIVIDUALS OF *NEMERITIS* EXPOSED TO THE ODOUR OF *MELIPHORA* FOR 48–72 HR AND THEN TO THAT OF *EPHESTIA* FOR 12–24 HR, WHEN TESTED IN THE OLFACTOMETER *MELIPHORA* AGAINST "BLANK"

Exp. no.	*Meliphora*	"Blank"	Total
1	4	4	8
2	2	4	6
3	5	2	7
4	5	2	7
5	3	5	8
6	4	2	6
7	14	12	26
8	15	13	28
9	16	14	30
10	17	13	30
11	10	18	28
12	13	8	21
13	7	5	12
14	18	14	32
15	15	12	27
16	22	17	39
17	11	8	19
18	13	12	25
19	9	9	18
20	14	8	22
21	13	10	23
Total	230 = 54·5 % S.E. ± 2·43 %	192 = 45·5 %	422

It will be seen by comparison with Tables II and VII that the proportion attracted to *Meliphora* has been considerably reduced, indeed it is not significantly different from the controls (Table I). From this one concludes that the effect produced by post-imaginal treatment in the "conditioner" is not so stable as that resulting from rearing on *Meliphora*.

Response of *Nemeritis* to its own odour

As recorded in the previous paper, super-parasitism is quite frequent in *Nemeritis* when confined in a small space with a limited number of hosts The efficiency of the parasite must, under certain conditions, be greatly diminished by this tendency, since never more than a single individual is able to come to maturity in one host It is known that some parasitic insects can avoid laying eggs in a host which has already been parasitized by a member of their own species Salt has brought forward evidence that this ability to avoid already parasitized hosts is (to some extent) dependent upon smell ovipositing females being repelled by the odour lingering on the surface of the host for some considerable time after oviposition Previous experiments on *Nemeritis*, although not specifically designed to this end, did not give evidence that any such repulsion was occurring To investigate this matter by means of the olfactometer is not easy, since as soon as any of the *Nemeritis* have entered the two traps the air stream in both arms will for the remainder of the experiment be contaminated by their odour Accordingly a modification of the apparatus was made (fig 3) whereby the trap itself is isolated from the stream of air, so that the presence of *Nemeritis* in the trap does not vitiate the rest of the experiment As shown in the diagram, the air stream is introduced at the side by means of a small jet through which the air passes direct into the Y-piece of the olfactometer tube Insects entering one of the arms

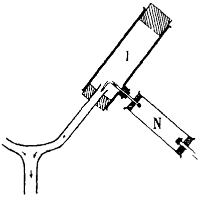

FIG 3 Modification of olfactometer to test response of *Nemeritis canescens* to odour of its own species N = tube containing living *Nemeritis* T = trap A similar jet is fitted to the other arm but tube N is left empty The insects which have passed through the olfactometer are trapped in the tube T out of the air stream and thus do not contaminate it with their odour Arrows indicate direction of air flow

of the olfactometer pass the aperture of the jet on their way into the trap and are there held in what is, as it were, a backwater of the stream, so that they do not contaminate it With the apparatus arranged in this way, the air coming down each arm is first rendered attractive by being passed through a tube containing a single *Ephestia* larva and in one case it is also passed through a chamber containing a large number of *Nemeritis* As shown in Table XIII the result of putting *Nemeritis* through the olfactometer under these conditions is that, far from being repelled by the odour of their own species, *Nemeritis* show a significant attraction to that arm of the instrument in which they encounter the odour of their kind It was found advisable to include the odour of *Ephestia* in each stream Without the presence of the *Ephestia* smell in both arms the experiments were hindered by the slow rate of progress of the *Nemeritis* which tended to accumulate in the region of the Y-piece and so render results less reliable

In the case of an insect with bisexual reproduction, this attraction to the odour of the species would be comprehensible as facilitating the meeting of the sexes In the case of an exclusively parthogenetic animal, such as *Nemeritis* apparently is an explanation is not easy to find * Most probably it is a survival from the period when males were of regular occurrence and still had some significance in reproduction Under present conditions it would seem that such a response would be disadvantageous to the species, in that it would tend to increase superparasitism One must remember however, that even though the insects tend to accumulate in the same general region, it does not follow that the same attraction is operative when oviposition is taking place It is quite conceivable that the ovipositing female would leave a distinctive odour upon the surface of the host, or food material of the host, which would have the effect of repelling another individual of the same species

It might be thought that this tendency to association with their own kind would render the previous results obtained by the use of the olfactometer less reliable, in that it would tend to accentuate the swing to the

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* It is possible that association between individuals of the same species may be of value in insects, as in birds, in providing exteroceptive stimuli The two groups are similar in that the evolutionary tendency of both may be considered as moving in the direction of perfection of instinctive behaviour rather than making the ready adaptation characteristic of the development of mammalian behaviour The companion or the mate may supply visual, olfactory or other stimuli necessary in order to live more or less fully (cf Fraser Darling, pp 103–5) But knowledge of insect psychology is still far too imperfect to allow of any profitable general discussion of such a subject at present

TABLE XIII EXPERIMENT TO SHOW THE RESPONSES OF 130 INDIVIDUALS OF *NEMERITIS* TO THE ODOUR OF THEIR OWN SPECIES

Exp no	Odour of *Nemeritis* in air stream	'Blank"	Total
1	12	12	24
2	16	9	25
3	22	24	46
4	14	10	24
5	11	13	24
6	28	22	50
7	12	7	19
8	9	12	21
9	12	13	25
10	21	14	35
11	14	9	23
12	14	10	24
13	15	10	25
14	14	7	21
15	16	9	25
16	19	10	29
17	18	14	32
18	14	6	20
19	10	8	18
20	23	11	34
21	11	13	24
22	13	8	21
Total	338 = 57 4 %	251 = 42 6 %	589

S E = ± 2 06 %

(N B In the case of exps 17–22 the odour of *Ephestia* was included in both air streams)

positive arm of the apparatus In practice, however, it seems that this cannot have any appreciable influence on the results, for in almost every experiment there are one or two individuals which pause in the region of the Y-piece, and of course even in the control experiments with *Ephestia* there are always a certain number that find their way into the trap on the negative side Consequently, throughout the duration of almost all the experiments, both air streams must have been contaminated with the smell of *Nemeritis* so that any effects due to this would cancel out

ATTRACTIVENESS OF THE WEB

The fact that oviposition takes place more readily if the host insect is within its cocoon, or hidden in flour contaminated with the silken webbing of the larvae, has been commented upon in the previous paper It was also shown that the stabbing reflex could readily be called forth by the odour which is retained by a surface over which the host larva has walked, or by the presence of the web of the larvae alone In fact, the web and faeces will often give rise to the stabbing reflex more readily than will a clean host larva itself Accordingly it was interesting to determine whether the same effect operates at a distance Whether, in fact, the odour of the web and faeces is more attractive to *Nemeritis* than that of the host itself To test this, individuals of *Nemeritis* were put through the olfactometer, *Ephestia* larvae being placed in one of the bait tubes, web and faecal material of *Ephestia* only in the other As will be seen from Table XIV, the results were conclusive and showed that, as far as odour at a distance is concerned, it is primarily the scent of the larvae themselves which is the attractant It appears that only where actual contact is involved does the web rival the larva itself as a stimulus

TABLE XIV EXPERIMENTS TO SHOW RESPONSES OF TWENTY-FIVE INDIVIDUALS OF UNCONDITIONED *NEMERITIS* REARED FROM *EPHESTIA* WHEN GIVEN THE CHOICE BETWEEN CLEAN *EPHESTIA* LARVAE AND WEB AND FAECES OF *EPHESTIA* LARVAE IN OLFACTOMETER

Exp no	Clean larva of *Ephestia*	Web and faeces of *Ephestia*	Total
1	9	6	15
2	10	5	15
3	11	7	18
4	14	4	18
5	13	4	17
6	11	7	18
7	10	8	18
8	13	6	19
9	11	4	15
10	8	7	15
Total	110 = 65 5%	58 = 34 5%	168
	S E = ±3 86%		

INHERITANCE OF ACQUIRED CHARACTERS

In the previous paper it was shown that rearing *Nemeritis* on *Meliphora* for eight generations had not resulted in any significant augmentation of the proportion choosing *Meliphora* as compared with the first generation In other words, the experiments hitherto performed gave no definite evidence of the inheritance of acquired characters Since that paper was published, rearing work has been continued up to the eleventh generation, still without observing any significant increase It was accordingly assumed that a Lamarckian effect was not operating and this particular line of investigation dropped

CORRECTION

In the previous paper, Table XIII contained an arithmetical error The calculations in this table were regarded as showing a significant difference between the responses of *Nemeritis* reared from *Ephestia* and those reared from *Meliphora*, when given the alternative *Ephestia*—"Blank" Actually on the figures given the differences are not significant, although further work has shown that the figures for *Ephestia* in the first line were distinctly on the low side Extension of this line of investigation has shown that a significant difference does in fact exist, although the previous figures published had not reached the level of significance A revised table (Table XV) is therefore given This confirms the results which were based in the last paper on insufficient figures, and shows that the final conclusions need not be modified in any way

TABLE XV FOR EXPLANATION SEE TEXT

	Ephestia *a*	"Blank" *b*	Total
Nemeritis ex *Ephestia*	569 = 90 9 %	57 = 9 1 %	626
Nemeritis ex *Meliphora*	298 = 84 5 %	55 = 15 5 %	353
Total	867	112	979

$$\lambda^2 = \frac{(ad-bc)^2 N}{(a+b)(c+d)(a+c)(b+d)} = 9\ 43 \qquad P < 0\ 01$$

DISCUSSION

The term "conditioned" is perhaps an unfortunate one Pavlov when he speaks of an "unconditioned reflex" clearly does not mean a reflex which has no conditions, he merely means an inborn or natural reflex, whereas a conditioned reflex is one that has a special or artificial condition

The term "reflex" is also open to certain objections and, as Laird has shown, is used by Pavlov in such a loose manner that it means nothing more than "response" or "reaction" Discussion of the exact connotation of the term "reflex" would, however, be out of place here, and the expression "conditioned", while unsatisfactory in some ways, has of course come to stay What interests us at the moment is how far the experiments here described can be regarded as examples of reflex conditioning in the strict sense of Pavlov

There are several striking examples of strict experimental conditioning of the responses of insects, which show how adaptable the nervous system of these animals is, and which indicate that essentially the same laws must be governing the establishment of their nervous responses as are operating in the case of vertebrates (viz the famous experiments of von Frisch on bees and those of Turner on the cockroach) The fact that in comparatively few experiments have olfactory responses been the subject of critical investigation does not really bear on the matter Indeed, very little has been done with olfactory responses even in vertebrates, the reasons being primarily the difficulty of control owing to the problem of adsorption and the difficulty of standardization of odours But what work has been done by Pavlov and others has shown that olfactory stimuli may be conditioned in exactly the same way as visual, tactile and auditory stimuli What is remarkable about the results here described is that although there is clearly no objection to referring to the process as "conditioning", the method whereby the conditioning is brought about appears very different from that employed in the classical work of Pavlov and his school In those experiments it was the simultaneous or nearly simultaneous presentation of two stimuli, A and B, the natural and the artificial, which by continual reinforcement resulted in the conditioning of the stimulus B Here there is no such simultaneous association It appears at first sight to be merely a sort of "becoming aware" of a new odour Although there do not seem to be on record any experiments showing exactly the same phenomenon, the striking observations and experiments by Forel and Miss Fielde on ants and the careful work of Brun bear very closely on the subject It will be remembered that the hostility of ants is apparently aroused by any ant odour which a given worker has not encountered individually As a result of this the mixing of two colonies of the same species of ants frequently results in combat to the death, and the same applies to the mixing of two or more species or genera If, however, individuals are taken from two nests in the pupal stage and are placed together in the formicary they will hatch out and live amicably together In this way it is possible to establish artificial associations or alliances composed of two or even three distinct genera The same result can be brought about by mixing adult workers provided the initial period of strife can be got over It appears from the most interesting accounts of Forel and others that after a period of hostility the ant as it were becomes reconciled to the new smell, or aware of a familiar component in it, and the hostile reactions which are at first called forth are thenceforward inhibited From the results of Miss Fielde it seems that the odour is "inherited" from the queen, who retains it unchanged throughout her life All workers reared from eggs produced by a given queen are familiar with and will accept, or in other words are "conditioned to", that particular odour The odour of the workers changes, however, as they advance in age, so that if workers from a single nest are isolated the change becomes great enough to evoke suspicion in about 40 days It follows from this that throughout the course of the long life of a worker ant the conditioning to olfactory stimuli must be gradually changing, although the basic response to the unchanging odour of the queen remains There are many experiments which confirm this, but space is lacking to discuss them here the reader is referred to Forel's famous work "Le Monde Social des Fourmis du Globe" and to the papers of Brun It is however worthy of note that Miss Fielde described some experiments with a mixed colony of three genera of ants--*Camponotus*, *Formica*, *Aphaenogaster*--reared from the pupal stage, which after living amicably for about 6 weeks were then separated into their constituent genera Six months later they fought to the death if mixed again, but if recently emerged workers from eggs of the original *Camponotus* queen were put in with the *Formica*, 7 months after the original separation, they were accepted In other words these *Formica* recognized the ant odour of another species once familiar to them after 7 months of separation

It seems clear, therefore, that the process which is taking place in the experiments with *Nemeritis* and that which is proceeding in the course of the life of an ants' nest, and in the experimental establishment of compound ant colonies, is essentially the same It is a sort of "becoming accustomed to" a new odour constituting part of the environment but not specifically associated with a particular unconditioned reflex

How then can such results be accounted for? It might at first sight be thought that it was merely a fatigue of the sense organs which was involved, but a moment's reflexion will show that this could not be the case, for even in the experiments with *Nemeritis* the duration of the effect is much too long To imagine that it could also explain Miss Fielde's results is absurd We must therefore seek for some way of bringing such phenomena into line with the classical work on conditioned reflexes There is an important difference between the observations of Forel and Miss Fielde, and the experiments here described In the one case a new stimulus at first arouses intense hostility, whereas in the other the new odour at first calls forth no response and is apparently unrecognized

As far as the *Nemeritis* experiments are concerned there seem to be two alternatives Firstly, an explanation might be sought on the lines of Pavlov's observations on the differentiation of two closely allied stimuli, on the assumption that the odour of *Meliphora* has some element in it which is also a constituent of *Ephestia* odour but which is masked by a neutral odour, and that only after a period of training is the parasite able to differentiate the two components and so respond to the familiar part of the *Meliphora* smell Such obscuring of one odour by another is well known to workers on olfactory responses, and it would seem might account for the primary lack of response to *Meliphora* Pavlov himself, when discussing the differentiation of two closely allied musical tones to one of which a conditioned reflex had become firmly established, states that it is possible to regard stimuli neighbouring on a definite positive conditioned stimulus as bearing two aspects, one of similarity to and the other of difference from, the positive conditioned stimulus On account of the element which is in common these neighbouring stimuli can act similarly to a positive conditioned one, it is the presence of the second factor of difference which determines a temporary investigatory reflex bringing about external inhibition of the excitatory effect It is thus noticed that when a new stimulus (the neighbouring musical tone) is tried for the first time the conditioned reflex which results is frequently much weaker than that obtained with the original conditioned stimulus On repetition of the stimulus of the neighbouring tone—always of course without re-enforcement, the effect increases until it becomes equal to that given by the original stimulus But subsequently on further repetition it begins to diminish, falling finally to a permanent zero

While the experiments with the odour of *Meliphora* might possibly be interpreted on some such lines, it is difficult to imagine that those with the cedar-wood oil could also yield to the same analysis, for it is improbable that there is any familiar element in the odour of the cedar wood oil and of course the theory fails entirely to account for the case of the ants It must however be admitted that the cedar wood oil experiments are unsatisfactory, in so far as the substance normally exercises a repellent effect and even the maximum of training only results in a diminution of this effect it does not produce a positive response

The second alternative involves the possibility that the new stimulus, whether or not associated with any one clear-cut reflex response, is in general associated with conditions of a more or less optimum or, at any rate, a satisfactory environment It does not seem at all surprising that an insect, or any other animal, should become attracted by an odour or other stimulus which has been associated over a considerable period with the environment in which it has lived securely The insects in the "conditioner" are associated with their own species to which they have a definite positive attraction, they are supplied with food in the form of honey and sugar or dried raisins they have ample water supply in the form of a moist pad, and the temperature and humidity are approximately at the optimum It does not seem very astonishing that after a period under such conditions they should show a positive response to the characteristic odour which has been associated with that environment, and if this is so, the attraction to *Meliphora* is not specifically an oviposition response If it were possible to find an "artificial" odour to which the untrained insect is completely indifferent and then as a result of training secure a positive response to that odour, we could regard this theory as well substantiated But unfortunately the prospects of obtaining such conditioning do not appear very bright In the first place it has so far proved impossible to find an odour which is absolutely neutral and there are the invariable difficulties of standardization of the stimulus with which all workers on olfaction are familiar The alternative line of attack would be to compare the responses of insects exposed to the given olfactory stimulus as a component of an optimum environment with those exposed to the same stimulus in an unfavourable or less favourable environment Unfortunately it is difficult in the present state of knowledge of insect physiology and ecology to devise an experiment which would give an unequivocal answer, and there the matter must for the moment be left But short of actual proof it seems that the balance is strongly in favour of this second hypothesis Not only does this account for the pre-imaginal conditioning which the other certainly would not do, but it also accounts admirably for the results of observations and experiments on the ants Moreover, it does not rule out the action of the first hypothesis in certain instances It seems that it would be of the greatest interest to test for the existence of such a "becoming aware" of a new odour as part of a generally favourable environment in a higher animal more suitable for exact experimental work If conditioning of this new type is generally occurring among insects it makes plain many strange anomalies concerning the effect of the pre-imaginal environment on certain of the responses of the adult, and it throws light on many remarkable instances of insects colonizing new hosts and on many cases of apparent adaptation to new environments

In conclusion, as a piece of pure speculation, perhaps we may consider for a moment the bearing of this subject of pre-imaginal olfactory conditioning on the problem of the origin of the Holometabola Among the

Exopterygota it is rare for there to be any sudden change in food or the environment generally, on the completion of the final moult Consequently the responses associated with egg laying are usually of a rather simple type and may, for example, consist of little more than a tendency to insert the eggs in soil or, in the case of phytophagous species, in or on the tissues of plants on which the insect happens to be feeding But one of the chief problems encountered in the course of evolution of the more advanced type of holometabolous life history must have been the adjustment of the oviposition reactions to ensure the placing of the eggs in the appropriate situation when the divergence between the larval and imaginal environments which is characteristic of the Endopterygota was being brought about Possibly the first and most fundamental step in this direction was the specialization of part of the general associative mechanism of the brain to form a special associative mechanism whereby the chief stimuli characteristic of the larval environment, probably in the first place odour, became specifically linked with the nervous reflex mechanism of oviposition Once this primary adjustment had been made the way was open to more and more specialization of the larval and adult feeding habits, resulting in the ever widening divergence which has resulted in the Endopterygota being the dominant insects that they are Although there is little doubt that many if not most of these responses are now inherent and natural reflexes it is not perhaps surprising if the same general associative mechanism is still present in ontogeny and able to manifest itself as in *Nemeritis* in the absence of the special stimuli provided by the presence of the normal host

The probable evolutionary importance of such conditioning in providing a first biological barrier separating the members of a species into different groups, in such a way that modifications of genetical origin would be conserved, has already been mentioned in the previous paper and need not be discussed again here

To indicate the importance of the study of physiological isolation it is only necessary to quote Dobzhansky who refers to "the appallingly insufficient attention that the problem of isolation has received in genetics" and expresses the opinion that the mode of origin of the various isolating mechanisms at work in nature is as yet little understood

SUMMARY

1 In a previous paper (Thorpe and Jones 1937) it was shown that the ichneumonid parasite *Nemeritis canescens* (Grav) can be induced to

develop a positive response to the odour of an abnormal host (*Meliphora grisella*) by rearing it artificially upon that host It was also shown that this effect was due, in part, to the influence operating during the larval life and, in part, to the contact of the newly emerged adult parasite with the host larvae In the latter case, if the parasites are closely confined with the host larvae, a good deal of tentative stabbing with the ovipositor takes place and a few eggs may be laid There is, therefore, an opportunity for an association to be established between the act of oviposition and the odour of the larvae In the former case, of course, no such association can be established, so that a conditioned response produced by influences during the larval life cannot be a simple reflex association with the act of oviposition, but is rather something in the nature of a "becoming aware" of a new factor as part of a favourable environment The only comparable behaviour known in insects appears to be that described by Forel, Fielde and others in various species of ants The primary object of the experiments described in the present paper was to elucidate the nature of the conditioning process in *Nemeritis*

2 Adult *Nemeritis* reared from the normal host (*Ephestia*) were confined immediately on emergence in an apparatus through which was pumped a stream of air which had passed over a number of living *Meliphora* larvae In this way all possibility of direct association between the odour of the host and the act of oviposition was ruled out Nevertheless, when tested in the olfactometer these insects showed just as effective a conditioning as did those actually confined in contact with the host

3 The reaction of *Nemeritis* to the odour of various chemical substances was examined Cedar-wood oil was found to be a convenient substance for the experiment The odour of this oil is repellent to the normal insect If however the *Nemeritis* is exposed to it in an air stream, as in the above experiment, a high degree of tolerance is produced without in any way injuring the animals

4 Within certain limits variation in the period of conditioning does not alter the intensity of the final effect Thus 24–36 hr in the conditioner is as effective as 6 days' exposure

5 The effect of conditioning both to *Meliphora* and to cedar wood oil is greatest immediately after the period of exposure On subsequent isolation it decreases rather irregularly and approaches zero after a period of approximately 10 days

6 There is no definite evidence that younger insects are more readily conditioned than older ones

7 It was shown that contact with *Ephestia* after conditioning to *Meliphora* reduces the attractiveness of the latter insect

8 A modification of the olfactometer was made in order to test the response of *Nemeritis* to the odour of its own species It was shown that the odour of *Nemeritis* exerts a slight attraction on its own kind

9 Previous observation showed that the parasite lays eggs more readily in larvae covered with webbing and faeces than in clean larvae Experiments in the olfactometer in which clean *Ephestia* larvae were offered as bait in one arm as against webbing and faeces without any larvae in the other, showed that this does not apply to perception at a distance As far as odour alone is concerned the larvae themselves are far more attractive to the parasite than is webbing and faeces

10 The experiments have given no support for the theory of the inheritance of acquired characters

11 Attention is drawn to an arithmetical error in a table published previously A corrected table is published

12 It is concluded that conditioning in *Nemeritis* is not a simple association between the experience of a given constituent of the environment (e g odour) and the calling forth of a single specialized response (e g oviposition) It is rather the association of the given constituent with a favourable environment as a whole not a particular element of it, giving rise in this way to a positive response which will tend to keep the parasite in the type of environment in which the host itself occurs It seems that a similar concept provides the only satisfactory basis on which to explain various well known observations on the behaviour of ants

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THE CROONIAN LECTURE

Processes of urine formation

BY A N RICHARDS

(*Lecture delivered* 30 *June* 1938—*MS received* 30 *September* 1938)

I know of no higher honour which can come to an American laboratory than that which is implied in the invitation to a member of its staff to deliver a Croonian lecture before the Royal Society The obligations which acceptance entails are heavy, particularly so in the present instance, inasmuch as the foundations of knowledge concerning the subject of which I am to speak have been laid by past fellows of this Society and its chief developments have been made by their successors In accordance with the terms of the Council's invitation, what I shall have to say is based upon a series of connected studies* which have been in progress in the Laboratory of Pharmacology of the University of Pennsylvania for more than twenty years and is the product of a succession of collaborations which it has been my privilege to enjoy

It seems necessary to begin with a description of the structures which compose the kidney In the latter half of the seventeenth century crude conceptions based upon gross observation of the structure of the kidney began to be corrected by more minute study The Florentine anatomist, Bellini, in 1662 showed that the striations which are apparent to the eye when the kidney is sectioned are not fibres, as had been supposed, but are actually minute tubes Four years later, Malpighi (1666) discovered that in the cortex, associated with Bellini's tubes, are minute globular structures ("glandulae") in which the finest divisions of the renal arteries appeared to terminate He could not demonstrate a connexion between these and the tubes, but from the deductions of reason became convinced that the two structures must be continuous, that urine is formed within the "glandulae" and conveyed into the pelvis of the kidney through the tubes This conception, accepted and developed by some of his successors in the following century (Schumlansky 1782), was emphatically denied by the foremost authorities of the first third of the nineteenth century

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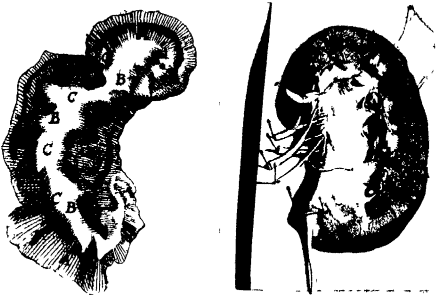
* Aided, since 1930, by generous grants from the Commonwealth Fund

Johannes Muller and his contemporary, E H Weber, searched for but could not find evidence of the continuity which Malpighi suggested Muller wrote in 1830, "Omnia quae Schumlansky de ductuum uriniferorum decursu exposuit, praestantissima sunt et mirum quam bene in tam parvis observationis praesidiis observata, sed falsissima est opinio de connexu ullo quopiam inter corpora Malpighiana sanguifera et ductuum uriniferorum fines " And in 1834, "Denn die Glomeruli seu corpora Malpighiana lassen sich nur von den Arterien aus injecieren, werden aber nie nach Injectionen der Harnkanalchen angefullt Sie sind uberhaupt blosse Receptacula des Bluts

"Die Quelle der Harnabsonderung sind die gewundenen Harnkanälchen selbst, welche die in Harn verwandelten Theile des Bluts ausscheiden "



A *B*

FIG 1 *A* Bellini's fig vii "Humani Renis medietas Pelvi, vasisque plurimis liberata, ut unio, & coitus tortuosus Renalium ductuum representetur " *B* A section of kidney, drawn by Bidloo (1685), showing Malpighi's "Glandulae" The "De Renibus ' of Malpighi was not illustrated From "Anatomia humani corporis", Plate 43 Amstelodami J à Someren

It was nine years later that Wm Bowman (1842), a young, recently elected Fellow, demonstrated to this Society preparations of kidneys from various animals which proved Malpighi to have been right By injecting lead acetate and potassium bichromate into the renal artery, he not only made clearly discernible the arrangement of the minute vessels which constitute the tuft (glomerulus) within the Malpighian body but also, by fortunate accident, showed that this structure is actually the expanded end of the uriniferous tubule For frequently the capillaries within the Malpighian body were ruptured by the force of the injection, and when this happened the lead chromate was found as a continuous mass, filling the free space within the Malpighian body and extending from this into the lumen of the tubule This identification of the unit of kidney structure constitutes the indispensable beginning of intelligent study of renal function

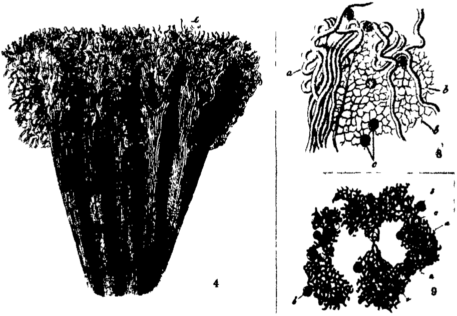


Fig 2 From Joh Muller's De Glandularum Structura", 1830, Tab xiv 4 "Pars renis Sciuri excisa, 8^{ies} diametro aucta, microscopio visa, accuratissime ad naturam delineata" 8 "Lamina subtilissima substantiae corticalis renis Sciuri excisa" 9 "Vascula sanguifera minima reticulata in substantia corticali renis humani"

Developments since Bowman's day have revealed greater complexities of this unit than he suspected The capillaries which constitute the glomerulus within the Malpighian body are not, as Bowman thought, "bare", "uncovered by any structure" each is invested by an extremely thin, supporting basement membrane and by an outer continuous covering of epithelial cells By the term glomerular membrane, we now mean the

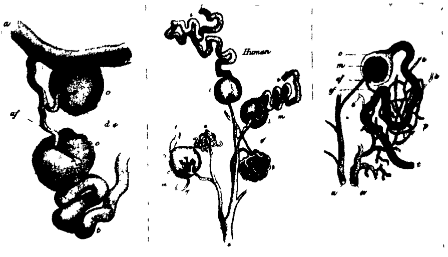


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FIG 3 Bowman's drawings 9 "From the human subject Two Malpighian bodies injected The tufts are burst and the fluid has escaped into the capsule In one case it has passed along the tube" 10 "From the human subject At (α) the injection has only partially filled the tuft At (β) it has entirely filled it without any extravasation At (γ) it has burst into the capsule and escaped along the tube (t), but has also filled the efferent vessel (ef) At (δ) and (ϵ) it has been extravasated and passed along the tube" 16 Plan of the renal circulation in mammalia"

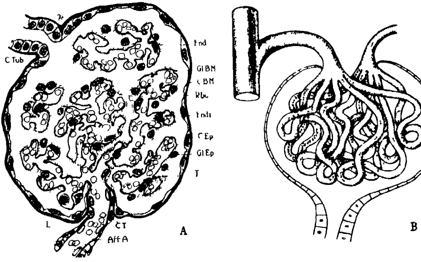


FIG 4 *A* Structure of the human glomerulus (McGregor) *GlEp* glomerular epithelium, *GlBM* glomerular basement membrane, *End* endothelium From *Amer J Pathol* 1929, **5**, 545 *B* Schema of the human glomerulus (Vimtrup) From *Amer J Anat* 1928, **41**, 123

wall of these blood vessels, composed of endothelium, connective tissue and epithelium

The tubule is far more tortuous in its course, its cellular components more various than Bowman could have thought Issuing from the Malpighian body is a short narrow segment, the neck, a series of turns, the proximal convolutions, a loop with straight limbs extending into the medulla, a series of distal loops or convolutions, finally, a short segment by which connexion is made with a collecting duct which delivers the products of many similar units into the pelvis of the kidney, thence into the ureter and bladder

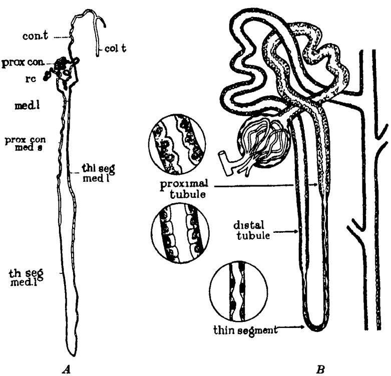
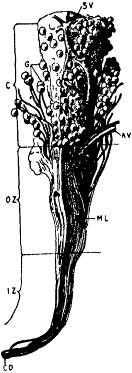


FIG 5 *A* A single tubule with its Malpighian body teased from a rabbit's kidney (Huber) From Cowdrey's 'Special Cytology", 2nd ed 1932, 2, 195 (New York, P Hoeber) *B* Schema of the mammalian nephron From Smith's "Physiology of the Kidney", 1937, p 6 (New York Oxford Univ Press)

The human kidney contains approximately a million of these units, which, though alike in design, differ with respect to lengths of the several segments These million are grouped into a dozen major divisions, the pyramids, and these into the groups which discharge through a common orifice into the renal pelvis Those subdivisions in turn are further divisible into "structural units", roughly pyramidal blocks, each consisting of about 150 tubules together with their Malpighian bodies and blood vessels Without gross inaccuracy we may imagine the human kidney as constructed of some six to eight thousand of such blocks arranged in a dozen major divisions

The blood supply of the kidney is remarkable because of its great volume and because of the peculiarity of its distribution The renal artery, one of the great branches of the aorta, is short and large in diameter Its divisions within the kidney proceed toward the cortex, there give off the finer branches from which arise short terminal twigs (afferent arterioles) which supply the glomerular capillaries enclosed within the Malpighian bodies The emergent efferent vessels divide into a profusion of capillaries which are distributed to the tubules The abundance of the arterial supply makes credible the statement that an amount of blood equal to more than five times the weight of the kidney may traverse its vessels in a minute, that all of the blood in the human body may pass through the renal vessels in as short a time as three or four minutes



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We have now to ask by what processes this assemblage of structures is capable of continually elaborating urine from blood in such varying amount and of such varying composition that despite the wide variations in bodily metabolism and the periodic intake of food, salts and fluid, the composition of blood plasma and tissue fluid, the "milieu interieur" of Claude Bernard, is held constant within those exceedingly narrow limits which are required for survival

Bowman outlined an imaginative conception, the first to be based upon demonstrable knowledge of the functional unit Impressed by the similarity which the form and arrangement of cells of the tubules bear to those of the digestive glands, he wrote, concerning the tubule "In extent of surface, internal structure and nature of its vascular net work, the membrane of the uriniferous tubule corresponds with that forming the secreting surface of other glands Hence it seems certain that this membrane is the part specially concerned in eliminating from blood the peculiar principles found in urine "

And concerning the Malpighian body "Why is so wonderful an apparatus placed at the extremity of each uriniferous tubule if not to furnish water to aid in the separation and solution of the urinous products from the epithelium of the tube?"

The fundamental relation of Bowman's anatomical discovery to understanding of function was at once recognized, most fruitfully by a young German colleague, Carl Ludwig at Marburg, later to become the most distinguished physiologist of his time Ludwig repeated Bowman's findings and in 1844 advanced a different interpretation

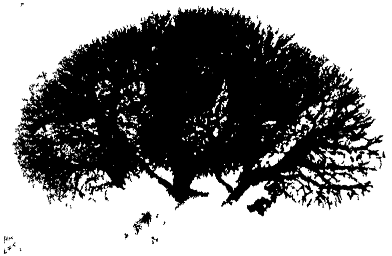

FIG 7 Roentgenogram of the arterial tree of the human kidney (Gross) From *J Med Res* 1917, 36, 336

The arrangement of the capillaries within the Malpighian body and their relation to the arteries which supply the kidney are such as to ensure high blood pressure within them In consequence, through their thin walls is expressed a filtrate from blood plasma which contains all the plasma constituents except those to which the capillary wall is impermeable, viz proteins, fats and such mineral constituents as may be combined with these The volume of filtrate is so great that it contains all of the solids of the urine which the kidney excretes As it flows through the lumina of the tubules it is subjected to the influence of the blood within the fine vessels surrounding them—blood which has been concentrated by loss of fluid in its passage through the glomeruli An "endosmotic current" is set up by which a large fraction of the filtrate is drawn back into the blood The fraction, not reabsorbed, is the urine which the kidney excretes

Ludwig speaks of his hypothesis as audacious Its audacity as well as its usefulness consisted in the definiteness with which the glomerular process was characterized as physical filtration, in the concept that *all* of the constituents of urine are separated from blood by the glomerular process, and in the novelty of the thought that in the elaboration of urine the transport of substances through the cells of the tubule is not, as the authorities of his time believed, in the direction blood to lumen of tubule, but rather in the direction lumen to blood

These two radically different conceptions, announced within the space of two years, neither based upon experiment but both the imaginative results of observations of structure, have set the questions which the great mass of subsequent experimental work has been designed to answer

Evidence which accumulated in the following thirty years upheld the filtration aspect of Ludwig's view and secured its general acceptance The rate of urine flow in animals was found to vary directly with such changes in the renal circulation as might be expected to change filtration pressure in the glomerular capillaries inhibition of the heart, haemorrhage or partial obstruction of the renal artery lowered renal blood pressure and decreased urine, ligature of great branches of the aorta or section of vasoconstrictor nerves to the kidney raised the renal blood pressure and increased urine

Ludwig's thought that the glomerular filtrate is reduced in volume in the tubules solely by processes of diffusion soon became unacceptable when it was found that the osmotic pressure of urine may far exceed that of blood To retain the reabsorption hypothesis it became necessary to postulate active, selective processes, operating within the tubule cells, undescribed save by the word "vital"

Later in the century (1874–86) more varied types of experimentation were introduced from which severe criticisms of Ludwig's view developed Heidenhain of Breslau (1874) wished to make visible the paths which a substance traverses in passing through the kidney from blood into urine He had no method for detecting normal urinary constituents in tissue sections, hence he chose to study the excretion of the dye indigo carmine, which, after injection, is, like urea, excreted in high concentration in urine and is recognizable in tissues by its colour

A rabbit's kidney, excised during the excretion of this dye, showed colour in the tubules, none in the Malpighian bodies After injection into a rabbit whose kidneys were excreting no urine because of low arterial blood pressure produced by section of the spinal cord, crystals of the dye were found in the lumina of the tubules These results proved to Heidenhain that the tubules and not the glomeruli were the structures which excreted indigo carmine

Heidenhain (1883) further pointed out that the volume of a blood filtrate sufficient to contain all of the urea which a man excretes in urine in a day must be of the order of 70 l , of this, according to Ludwig, 68 l must be reabsorbed He was unable to conceive that enough blood flowed through the kidneys in a day to yield such a volume of filtrate Chiefly because of these experiments and criticisms and the skill of their exposition, the Bowman conception of secretion of "urinous products" by the epithelium of the tube was reinstated, Ludwig's theory abandoned, the Bowman-Heidenhain theory became accepted doctrine for more than a generation

But at about the opening of this century, new evidence began to appear which accorded so well with the demands of the filtration-reabsorption theory that a number of physiologists were persuaded that its rejection was unwarranted A confusing controversy developed which continued actively during two decades and even now is scarcely ended I shall make no attempt to give any of its details or to analyse opposing arguments That has been done with extraordinary competence by Professor Cushny in 1917 and again in 1926 The division of opinion may be illustrated by the fact that within the membership of this Society at that time, Starling, Cushny and Bainbridge were convinced of the validity of the filtration-reabsorption hypothesis, whereas Leonard Hill, A B Macallum and T Gregor Brodie with equal or greater conviction denied it The thesis of Professor Brodie's Croonian Lecture of 1911 was a new and ingenious conception of glomerular function, formulated because of his belief in the untenability of filtration

The source of the difficulty is obvious enough On one side of the complex renal structure is blood, on the other side urine The composition of the blood, its pressure and rate of flow, and the ureteral pressure against which urine is excreted could be altered at will The effects of such changes upon volume and composition of urine could be measured There observation on the mammalian kidney for the most part stopped Decisions concerning the intermediate processes within the kidney were inferences, the quality of which was largely dependent upon imaginative insight, intuition and perhaps prejudice

In such a situation it was exceedingly difficult for one interested in the effects of drugs to explain the action of substances which produce diuresis

A disciple of Heidenhain was free to regard caffeine, for example, as a stimulant to the secreting epithelium of the tubes, a follower of Ludwig and Cushny could argue with equal plausibility that the known dilating effect of caffeine upon renal blood vessels caused increased glomerular filtration

It was because of this dilemma that in 1915 O H Plant and I undertook an experiment by which we hoped to learn whether caffeine could produce diuresis when the results of its vascular action were minimized or excluded By an artificial device (Richards and Drinker 1915) the flow of blood through a rabbit's kidney was kept constant before and during the action of that drug Somewhat to our surprise, diuresis resulted and we seemed obliged to regard the action as independent of the circulatory effects and as indicative of secretion That proved to be a superficial interpretation and has been superseded, but the experiment was useful in that it pointed the way to a decision concerning a far more fundamental question which, raised by Heidenhain years before (1883), had been the chief element in his belief that the glomerulus is not a filter

The question may be stated thus If the glomerulus is a filter, any measure which increases blood pressure in the glomerular capillaries may be expected to increase urine flow Two such measures have been tried, increase in aortic pressure and partial obstruction of the renal vein The first increases urine, but the second diminishes it The effective difference between them, according to Heidenhain, lies in the fact that the velocity of blood flow through the glomerulus is increased when aortic pressure rises, decreased when venous outflow is retarded Urine formation varies, therefore, not with height of glomerular blood pressure but with velocity of glomerular blood flow These facts, said Heidenhain, contradict the pressure hypothesis in the most decisive manner The epithelium of the glomerulus is a secreting structure, the activity of which depends upon its state of nutrition and upon the rate at which the materials for its activity are supplied to it

The method used in studying the effects of caffeine promised the possibility of submitting this question to more searching experimental test The intake tube of the perfusion pump was connected with a rabbit's carotid artery, the delivery tube with the artery of one kidney Flow of blood through the kidney, maintained by the pump, was constant, blood pressure in the renal vessels was varied by stimulation of their constrictor nerve supply, by the addition of small amounts of adrenalin to the perfusing blood and by gentle compression of the renal vein The results were decisive, rise in blood pressure produced by each of these means was consistently paralleled by increase in urine flow (Richards and Plant 1922*a*)

These results were important for us not only because they supplied a refutation of one of the most cogent of the objections which Heidenhain had raised against the view that the kidney is a filter, but because of another item of information which the method yielded

There is only one part of the vascular apparatus of the kidney in which a filtrate, expressed from the blood, can conceivably be delivered directly into the lumen of the tubule, that is the Malpighian body On this point Bowman, Ludwig and Heidenhain were agreed Hence the effective rise in pressure in the experiments just described must have occurred within the glomerular capillaries In the case of adrenalin it seemed necessary to believe that the rise in glomerular capillary pressure which had caused more urine to flow was the result of constriction of the efferent vessel of the glomerulus Constriction of afferent vessels could not prevent the pump from delivering blood in constant volume to the glomeruli, rise of pressure in the glomeruli could have been produced only by narrowing of the calibre of the glomerular outlets

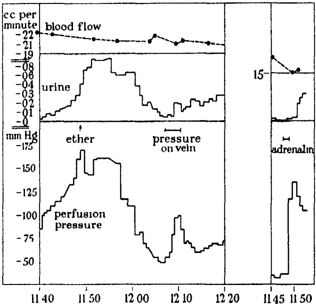

FIG 8 Effect of change in perfusion pressure upon urine formation by the rabbit's kidney, perfusion flow being kept constant The most conspicuous rise in pressure was due to renal vasoconstriction which resulted from diminished depth of anaesthesia

To test this conclusion, the kidney was enclosed in an oncometer and change in its volume, produced by adding adrenalin to the perfusing blood, recorded The result showed that coincident with rise in perfusion pressure adrenalin caused a slight swelling of the whole kidney (Richards and Plant 1922*b*)

This coincidence of vasoconstriction and vasodilatation, the former exhibited by rise in perfusion pressure, the latter by swelling of the kidney —a coincidence not encounterable in similar experiments with other tissues —could be ascribed only to passive distension of the glomerular capillaries produced by the narrowing of contractile vessels distal to them * In a word, the efferent vessel is an arteriole, the calibre of which is subject to hormonal and nervous control, and is therefore a factor in the regulation of glomerular pressure, hence in gomerular urine formation

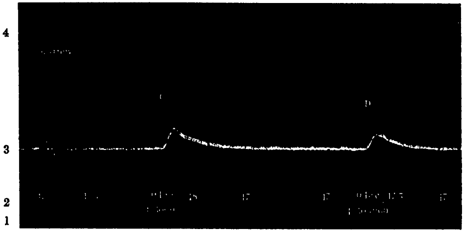

FIG 9 Effect of adrenalin upon perfusion pressure and kidney volume 1 Time in seconds 2 Rate of perfusion (time required for 4 c c of blood to flow from the renal vein) 3 Perfusion pressure 4 Volume of kidney From *Amer J Physiol* 1922, 59, 188.

It was to test further the validity of these deductions that we had recourse to the frog's kidney, hoping to find it to be sufficiently translucent to permit direct microscopic observation and measurement of its glomerular vessels In work with C F Schmidt (1924) this hope was realized and eventually perfusion experiments were made, identical in plan with those made with the rabbit's kidney (Richards, Barnwell and Bradley 1927) The addition of traces of adrenalin to blood with which the kidney was perfused at constant rate caused enlargement of the tuft of glomerular capillaries (fig 10) which could be attributed to nothing else than constriction of the efferent vessel

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* Histological evidence of the existence of contractile elements in the wall of the efferent vessel was later obtained by Bensley (1929)

This succession of results furnished objective evidence that glomerular capillary pressure is subject to change not only because of changes in aortic pressure and in the calibre of the afferent arteries and arterioles but also because of changes in state of the efferent vessel If the glomerulus is a filter, they permit us to think of a mechanism within the kidney itself for the fine adjustment of filtration pressure which is independent of changes in the systematic circulation as a whole, an adjustment which must be taken into account in attempts to correlate changes in urine flow with changes in arterial blood pressure or in renal blood flow

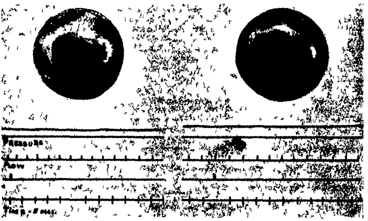

FIG 10 Photomicrographs, enlarged and retouched, of a frog's glomerulus during perfusion of the kidney at constant rate with frog's blood before and during the action of added adrenalin concentration 1 500,000

The experiments with frogs served also to introduce to us a hitherto neglected method of approach to problems in renal physiology, that of direct microscopic observation and manipulation of the amphibian kidney during life A year was spent by Dr Schmidt and myself in perfecting technique of visualization and in describing phenomena of the circulation in the kidney under varied conditions It was seen, for example, that the number of functioning glomeruli and of capillary loops in a single glomerulus were changing factors, that during renal vasoconstriction the number of active glomeruli decreased, while under the influence of diuretic substances more glomeruli came into action It became obvious that if the glomeruli are filters the organism possesses means of increasing or decreasing the extent of the filtering bed

A little later, thanks to acquaintance with the work of Robert Chambers and a suggestion from my then colleague, J T Wearn, a plan was initiated for withdrawing fluid from single renal units in the hope that it could be subjected to chemical tests and its composition compared with the blood plasma from which it was derived Glomerular fluid was first studied because it represents the first stage in urine formation and because the Malpighian body in which it is formed is most easily accessible (Wearn and Richards 1924)

A pipette was made, consisting of a glass stopcock tube into one end of which was cemented a slender quartz tip, terminating in a sharp, bevelled point, 7–12μ in diameter Tube and tip were filled with mercury from an attached levelling bulb With the aid of a microscope and mechanical manipulator the sharp point was thrust through Bowman's capsule, usually without touching the tuft of capillary blood vessels within it Fluid was collected against positive pressures determined by the height of the levelling bulb From a single glomerulus Dr Wearn collected as much as 4 cu mm in an hour, usually the amounts were 1 cu mm or less per hour

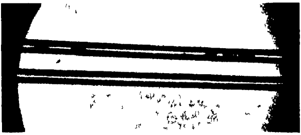

FIG 11 Result of Benedict's test for glucose in frog's glomerular urine (upper tube) and in bladder urine (lower tube) From *Amer J Physiol* 1924, **71**, 222

When these specimens of glomerular fluid were delivered into glass or quartz capillary tubes and mixed with appropriate reagents they were found to be faintly alkaline in reaction, and to contain no protein or only the faintest traces of it, they gave unmistakable tests for glucose, chloride, urea and potassium Bladder urine, simultaneously formed, was acid, contained no glucose and no chloride When the animal had been injected with indigo carmine or with phenol red before the beginning of a fluid collection, the dye was found to be present in the glomerular fluid

This qualitative information agreed well with the Ludwig concept of glomerular filtration, it showed that the glomerular membrane in frogs is impermeable to the plasma proteins but is permeable to every diffusible constituent of plasma for which we were able to test It appeared that

Heidenhain might have been wrong in denying that indigo carmine is excreted by rabbits through the glomeruli

The most significant fact, however, which these tests demonstrated was that the glomerular urine contained glucose and chloride, whereas the fully elaborated urine which issued from the kidney contained neither The glomerulus allows these essential constituents of blood plasma to escape, they are restored to the blood by the tubule

Knowledge that the glomerular urine bears qualitative resemblance to a protein-free filtrate from plasma made evident the necessity for a decision concerning the height of the glomerular capillary blood pressure

In 1899, Professor Starling had published evidence to show that, in dogs, urine ceases to be formed when aortic pressure sinks to a level somewhat above the value which his measurements had assigned to the osmotic pressure discovered by him to be exerted by the plasma proteins Arguing that the difference between the two values, some 10 mm Hg, represented the fall in pressure which must occur between the aorta and the glomerular capillaries, he held that the approximation of the two values constitutes evidence for the existence of a filtration factor in urine formation His views were contested by Leonard Hill (1906) and fifteen years later Hill and McQueen (1921) published measurements which appeared to support Professor Hill's earlier contentions They independently developed the method of direct observation of the living frog's kidney, compressed a portion of the kidney between two transparent surfaces, measured minimal pressure required to produce visible retardation of blood flow in the glomerular capillaries and decided that this was equal to the glomerular capillary pressure It was found to be 5–10 cm of water –somewhat less than the colloid osmotic pressure of frog's plasma proteins and hence inadequate for the separation of a protein-free filtrate from plasma Since the validity of their method was open to the criticism that even a slight impediment to venous blood flow must produce retardation in glomerular flow, and since the technique of glomerular puncture appeared to offer another method to which this criticism did not apply, it seemed necessary to re-examine the question This was done by J M Hayman (1927) in our laboratory, who adopted a procedure the principle of which is the same as that of the Riva-Rocci method of estimating pressure in the brachial artery of man

The puncturing pipette, filled with salt solution instead of mercury, was connected with pressure measuring and pressure adjusting devices After introducing the point of the pipette into the space within Bowman's capsule, the neck of the tubule was blocked by a compressing rod and pressure within the capsule raised to the height necessary to stop flow of blood in some capillaries of the glomerular tuft but not in others This pressure was accepted as the mean pressure within the capillary tuft Of 181 such measurements, only eleven fell below 10 cm of water The average of all determinations of glomerular capillary pressure was 20 2 cm H_2O, 54 % of the average aortic pressure Hayman's method was later applied by H L White (1929) to Necturi and the results confirmed We regard these measurements as proof that normally in frogs and Necturi there is an ample head of pressure in the glomerular capillaries for the separation of a protein-free plasma filtrate through their walls.

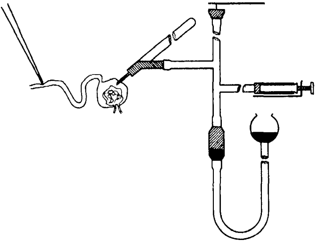

FIG 12 Diagram of Hayman's method of measuring glomerular capillary pressure From *Amer J Physiol* 1927, 79 391

Proof of qualitative resemblance between frog's glomerular urine and a plasma filtrate excited the desire for more precise, quantitative information The chief difficulty consisted in the minuteness of the volumes of fluid obtainable, since methods for quantitative analysis of less than a cubic millimetre of fluid were not directly available Attempts to estimate freezing points were futile The results of a first series of nephelometric determinations of Cl by the method of T W Richards were later found to be wrong (Wearn and Richards, 1925), in a second, the results were ambiguous (Freeman, Livingston and Richards 1930) Presently we found that accurate determinations could be made of the colour intensity of a minute sample of a coloured fluid contained in a capillary tube by naked-eye comparison with standard solutions in tubes of the same diameter

This led not only to quantitative estimations of the concentrations in which injected dyes are excreted in the glomerular urine (Richards and Walker 1930), but also to the adaptation to our requirements of accepted colorimetric methods for determining uric acid (Bordley and Richards 1933), glucose (Walker and Reisinger 1933), inorganic phosphate (Walker 1933), and creatinine (Bordley, Hendrix and Richards 1933) A new and highly accurate colorimetric method for chloride (Westfall, Findley and Richards 1934) was devised applicable to as little as 0 001 mg NaCl *

*p*H was determined colorimetrically with phenol red within 0 02 of a *p*H unit (Montgomery 1935)

H L White (1928) showed the feasibility of adapting Barger's capillary method of molecular weight determination to comparison of the vapour pressures of plasma and glomerular urine—an adaptation which was fruitfully utilized by Walker (1930) Leonard Bayliss (1930) constructed a cell with which he measured reliably the electrical conductivity of 0 5 cu mm of fluid A M Walker (Walker and Elsom 1931) developed a technique for the gasometric estimation of the nitrogen in as little as 0 006 μg of urea contained in 0 05 cu mm of fluid Pierce and Montgomery (1935) constructed a quinhydrone electrode by which they measured the *p*H of as little as 0 1 cu mm of fluid

As each of these methods was developed and the limits of its accuracy discovered it was applied to the determination of the corresponding constituent or constituents of simultaneously collected glomerular urine and blood plasma from frogs and Necturi The data which thus accumulated between 1930 and 1935 provide a fairly comprehensive picture of the quantitative likeness of the glomerular urine and the blood plasma or an ultrafiltrate from it obtained from these animals They are assembled in the chart shown in fig 13

In the majority of the eleven categories of results the distribution of the values of glomerular urine in relation to those of blood plasma is such as to leave no doubt concerning identity of composition of the two fluids The Cl concentration of glomerular urine seems consistently to be slightly greater than that of plasma This difference is explainable as a manifestation of the Gibbs-Donnan effect, which has been calculated to account for a difference of about 3 % between the chloride of frog's plasma and that of a protein-free filtrate from it (Wearn and Richards 1924) And since

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* Ekehorn (1931) at Oxford, developed a microtitration method for the determination of chloride and made five analyses of frog's glomerular urine, the results showed identity with plasma His method seemed so appallingly difficult that we were afraid to undertake it

sodium chloride is the most abundant electrolyte of frog's plasma, the same explanation can account for a similar difference in total electrolytes The average concentration of glucose in glomerular urine appears to be slightly less than that of plasma This may perhaps mean that a small fraction of glucose which passes through the glomerular membrane is utilized by its cells

The last two columns show the glomerular excretion of substances foreign to the body, injected before the beginning of an experiment The wide dispersion of the results with phenol red requires comment This

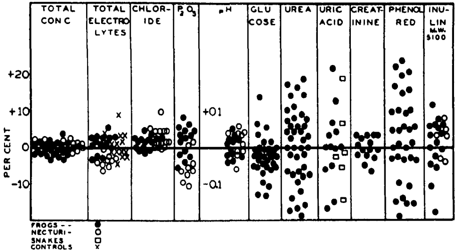

FIG 13 Comparisons of glomerular urine and blood plasma The results are shown as percentage differences between glomerular urine and plasma

dye when present in blood is partially combined with the plasma proteins and hence is not filtrable The combined fraction varies with the protein content of the plasma and was found to amount to from 16 to 40% of the total We were unable to obtain ultrafiltrates from the small samples of blood plasma obtained in each experiment and hence in charting the results have applied a correction of 28% to each plasma phenol red value While the individual results are therefore inaccurately charted, the approximately equal dispersion of the glomerular values above and below the plasma values gives confidence in the soundness of the conclusion that the concentration of phenol red in glomerular urine is the same as that of the free phenol red in the plasma

The results with inulin are especially significant because of the size of its molecule This polysaccharide, believed to consist of a chain of some

32 fructose groups (Haworth, Hirst and Percival 1932), has a molecular weight of about 5000 (Haworth, Hirst and Percival 1932, Westfall and Landis 1936) and a diffusion coefficient equivalent to that of a spherical molecule with a molecular weight of 15,000 (Bunim, Smith and Smith 1937) It does not combine with plasma constituents, is not decomposed by enzymes of the animal body and can be quantitatively determined after hydrolysis with acid by the same method as that used in the determination of glucose The results show that inulin is present in the glomerular urine of frogs in the same concentration as in plasma (Hendrix, Westfall and Richards 1936), it passes through the membrane at the same rate as water or such small solutes as urea or chloride Hence we draw the conclusion that the glomerular urine escapes through pores or passages in the glomerular membrane of dimensions so much larger than those of the molecules of any of the normal, diffusible solutes of plasma that it becomes inconceivable that any other than a physical process is concerned, and from the fact that two substances having as widely different diffusion coefficients as urea and inulin (Bunim, Smith and Smith 1937) pass through at the same rate and that the same as the rate of the water in which they are dissolved, we must conclude that diffusion plays no part, as some have thought, in determining the composition of glomerular urine, filtration is the only process concerned

These conclusions are fortified and the definition of the permeability of the glomerular membrane made more precise by study of the glomerular excretion of two other substances having larger molecules, viz crystallized egg albumin and crystallized horse serum albumin The tests were made by perfusing the frog's kidney through its arteries with oxygenated Ringer's solution to which the protein had been added Egg albumin was found in the collected glomerular fluid in abundant amount, its concentration, apparently, somewhat less than that in the perfusion fluid Horse serum albumin was not found in the glomerular fluid, even in traces From these results we might describe the mesh of the greater part of the glomerular membrane as wide enough to permit molecules of 4 mμ in diameter to pass but nowhere wide enough to allow passage of those of diameters as great as 6–7 mμ

According to Ludwig's concept the volume of the glomerular filtrate is so large that all of the solutes of the urine are contained in it It is appropriate to inquire into the relative proportions of glomerular filtrate and excreted urine in Amphibia

I have stated that in exceptional experiments as much as 4 cu mm of glomerular urine had been collected from a single glomerulus in an hour, more commonly the rate was 1 cu mm /hr or less One kidney of a frog such as those we used contains about 2000 glomeruli It is conceivable therefore that the two kidneys might form glomerular filtrate at rates up to 16 c c /hr In experiments designed to give measurements of the highest rates at which urine may be formed in normal frogs of the same size and species as those used in the glomerular puncture experiments the maximum rate was 2 5 c c /hr In frogs subjected to the operative attack necessary for exposure of the kidney the rates are far lower than this From such observations I have little hesitancy in asserting that in frogs the amount of urine excreted by the kidney is less than the amount of filtrate formed in the glomeruli

Experiments of an entirely different sort have yielded the same conclusion In the frog's kidney the glomeruli are supplied with blood by way of the renal arteries, the tubules receive blood not only from the branchings of the renal arteries but also from those of the renal-portal vein as well This arrangement makes it possible in perfusion experiments to supply the tubules through the renal portal vein with fluid which does not gain access to the glomeruli (Cullis 1906) In such an experiment, if inulin is contained in the perfusing fluid which reaches the tubules only, none of it gets into the urine We conclude therefore that the only renal pathway of the excretion of inulin by intact frogs is the glomeruli, and when we found that the concentration of inulin in urine collected during its excretion is as much as 8 to 15 times as great as the concentration of inulin in the blood plasma during the same time, we were obliged to conclude that the volume of glomerular filtrate was from 8 to 15 times greater than the volume of the excreted urine

The urine of amphibia is highly hypotonic in comparison with blood plasma It contains little or no chloride, no glucose, a trace only of bicarbonate, these being the three diffusible constituents of plasma which are present in highest concentrations Hence the evidence that glomerular urine is a plasma filtrate and is separated in amount far larger than that of the urine which the kidney excretes compels us to believe that the most conspicuous processes to which the glomerular filtrate is subjected in its passage through the tubules are processes of reabsorption The methods which yielded specimens of glomerular urine were adapted to the collection of fluid from different levels of the tubules, and as a result a partial description can be given of the succession of changes which the glomerular filtrate undergoes within the uriniferous tubule

The technique of collecting fluid from known levels of a tubule is more difficult than that of collecting from a glomerulus (Richards and Walker

1937) The lumen is much narrower, only portions of a single tubule are visible and the loops of several tubules appear so confusingly intertwined as to make certain identification of the different parts of one impossible by inspection only An essential requirement was a method for measuring the distance within the tubule which the collected fluid had traversed from the beginning of the segment from which it was taken to the site of its collection For this purpose, after the fluid collection was completed, indian ink was injected into the lumen of the tubule, the kidney quickly excised, dehydrated, cleared and a scale drawing made of the entire nephron (Kempton 1937) The puncture hole could usually be seen in the cleared preparation and the distances from it to the ends of the segment measured on the drawing

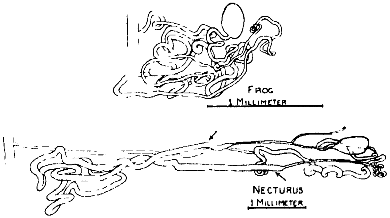

FIG 14 Scale drawings of injected tubules of frog and *Necturus*

It was essential also that the collected fluid should not be contaminated with fluid drawn back into the pipette from parts of the tubule distal to the site of puncture One way of avoiding this was by keeping the level of the mercury in the levelling bulb slightly above the level of the kidney, another by establishing a blockade of the tubule immediately distal to the point of the pipette, this was done by depositing a small globule of mercury or of coloured oil within the lumen

In some experiments it was necessary to perfuse a known solution through the lumen of a selected tubular segment in order to learn what would happen to one or another of its constituents Such perfusion of the proximal segment or a portion of it was effected by inserting two pipettes, one at each end of the segment to be studied, after glomerular function had been abolished by packing the space within Bowman's capsule full with mercury Fluid was then allowed to flow in from the proximally placed pipette at any desired rate and collected in the distally placed pipette

Because of the larger dimensions and less tortuous course of the tubules of *Necturus* this species was used in the first experiments and in the majority of all The more difficult task of collecting tubule fluid from frogs was accomplished in enough instances to show that the main features of tubular activity are alike in the two species

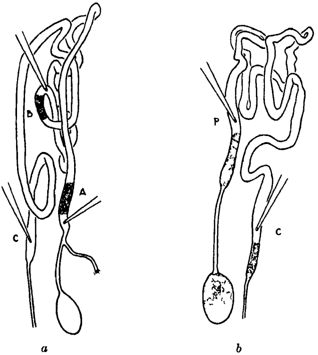

FIG 15 *a* The insertion of a micropipette for the collection of tubule fluid from the beginning (*A*), the middle (*B*) and the end (*C*) of a proximal convoluted tubule of *Necturus* Stippling at (*A*) represents blockage of the lumen by a globule of mercury, at (*C*), by a column of coloured oil *b* Micropipettes inserted for perfusing the lumen of a single proximal convoluted tubule of *Necturus* Stippling represents injected mercury by which glomerular filtration is abolished and the distal end of the segment blocked From *Amer J Physiol* 1937, **118**, 114, 116

In each experiment the composition of the collected tubular fluid was compared with that of blood plasma from the same animal, obtained either as two samples, one at the beginning and one at the end of the tubule fluid collection, or as a single sample taken midway through the collection Proof of the identity of glomerular urine and plasma filtrate made it permissible to use the plasma analyses as representative of the glomerular urine from which the tubule fluid is elaborated

The results are charted to show the differences between tubule fluid and plasma which develop during the passage of the glomerular filtrate through various fractions of the length of the two main divisions of the tubule

Fig 16 shows the results of the comparison of vapour pressure (total molecular concentration) and of chloride concentration (Walker, Hudson, Findley and Richards 1937) It is at once apparent that significant change does not occur until the glomerular filtrate has traversed the entire length of the proximal and intermediate segments In the distal convoluted tubule selective reabsorption of chloride occurs, this is the chief reason for the hypotonicity of amphibian urine These data appear to supply adequate refutation of the arguments of Macallum and Benson (1909) and Brodie (1914) that reabsorptive processes in the tubule cannot be sufficiently effective to account for the high dilution of the urine which is excreted by man after copious water drinking

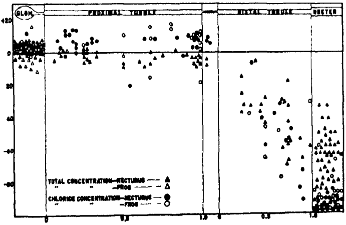

Fig 16 Chart showing differences between blood plasma and fluid collected from various levels of the renal tubules with respect to total concentration and Cl concentration Sites of collection of tubule fluid can be identified by reference to the diagrammatic tubule Zero on the ordinate represents plasma For chlorides, figures above and below represent percentage differences from plasma, for total concentration, they represent the micrometer scale divisions (each 5μ) by which the 2 mm capillary column of tubule fluid increased (+) or decreased (−) during 48 hr equilibration against plasma From *Amer J Physiol* 1937, **118**, 125

Whether the lack of close agreement between the values of total concentration and chloride concentration in fluid from the proximal tubule represents something other than experimental and analytical errors or not we are unable to say The difference does not disappear when account is taken of the presence of protein in the plasma and its absence from the tubule fluid, of the Donnan effect, and of the reabsorption of glucose which was found to occur in the proximal tubule (*vide infra*)

Fig 17 shows that the change in reaction which the glomerular filtrate undergoes in its passage through the tubule occurs in the distal, not in the proximal segment (Montgomery and Pierce 1937) This result is opposed to that published by Ellinger and Hirt (1929, 1930), who believe, from observations of the colour of fluorescein in different segments of the tubules of frogs, that acidification occurs in the proximal tubules We have not used fluorescein but our confidence in the correctness of the results shown

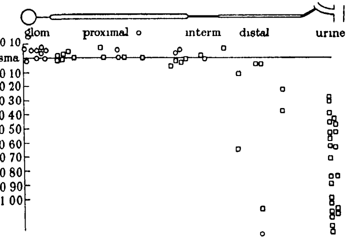

FIG 17 *p*H determinations by Montgomery and Pierce (1937) of fluid collected from different levels of the tubule Ordinates are fractions of a *p*H unit From *Amer J Med Sci* 1935, **190**, 742

here is strengthened by observations of another type It is easy to inject a solution of an indicator dye, phenol red, of *p*H 7 4 into the capsule of Bowman, to watch its progress through the visible loops of the tubule and to halt its progress at any desired level by compressing the surface of the kidney with a microscopic rod Such observations have repeatedly shown that the yellow colour, indicative of *p*H 7 0 or lower does not develop in the proximal tubule no matter how long (within 20 min) the fluid column is made to remain in it When, however, it is allowed to flow on into the distal segment, the change to yellow is almost instantaneous Furthermore, when by rapid injection the lumen of the distal segment is filled with a buffered solution of the dye of *p*H 7 4–7 6, and the fluid column made to remain stationary within it, the yellow colour develops only within a restricted portion of the distal tubule (Montgomery and Pierce 1937) The site of this restricted portion is such as to indicate that the acidifying structures are localized in an area roughly corresponding to the middle third of the distal convoluted segment (fig 18)

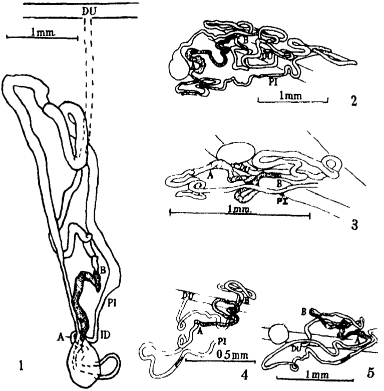

FIG 18 Location and extent of the acidifying segment of the distal convoluted tubule 1, an entire nephron of *Necturus*, 2, of frog 3–5, distal tubules of frogs *A* represents the acid change point in each, *A–B* (stippled) the entire acidifying segment *PI* = junction of proximal and intermediate segments, *ID* = junction of intermediate and distal segments, *DU* = junction of distal tubule with ureter From *Amer J Physiol* 1937, **118**, 148

Fig 19 shows the fate of the glucose of the glomerular filtrate in the tubule (Walker and Hudson 1937*a*) Its concentration begins to diminish as soon as it enters the proximal segment, is reduced by one-half when it has traversed one-quarter of its length and approaches zero or its maximum reduction when half of the length of the proximal segment has been passed When known glucose solutions were perfused through the distal half of the proximal segment similar reduction in the glucose concentration occurred Reabsorption of glucose is therefore a function of the entire proximal segment However, when glucose solutions were perfused through the distal convoluted segment no such reduction in glucose concentration took place Hence it is evident that it is only from the proximal convoluted tubule that filtered glucose is selectively restored to the blood

For nearly fifty years it has been known that the glucoside, phlorhizin, injected into laboratory animals or man, causes the kidney to excrete

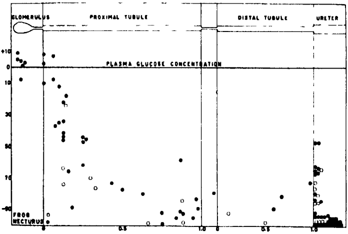

FIG 19 Chart showing percentage differences between glucose concentration of blood plasma and fluid collected from various levels of the renal tubules of normal Necturi and frogs Zero on the ordinate represents plasma From *Amer J Physiol* 1937, **118**, 133

glucose The effect has been variously ascribed to action on the glomerulus, to secretion of glucose by the tubule, to abolition of the capacity of the tubule to effect the reabsorption of glucose The methods used in identifying the course of glucose through the normal amphibian kidney have also been applied to those poisoned with phlorhizin No effect whatever could be detected on its passage through the glomerulus, the glucose concentration of the glomerular urine did not differ significantly from that in the plasma (Walker and Reisinger 1933) During the passage of the glomerular filtrate through the proximal convoluted tubule, however, its glucose concentration does not undergo progressive diminution, on the contrary, the concentration attained at the distal end of the proximal segment is on the average 25 % higher than the concentration in the blood plasma (Walker and Hudson 1937*a*) (fig 20) Since no other segment of the tubule possesses the capacity of effecting the selective reabsorption of glucose it seems obvious that the action of phlorhizin in inhibiting this capacity is confined to the proximal convoluted segment

This information concerning the retention of glucose within the tubule of the phlorhizinized kidney throws additional light, also, upon the question of the tubular reabsorption of the water of the glomerular filtrate We believe that the sole pathway by which glucose enters the lumen of the tubule is the glomerulus, neither in the normal nor in the phlorhizinized animal is it secreted into the lumen by the cells of the tubule If this is true, then the increase in concentration which glucose undergoes within the tubule can be due only to the reabsorption of water, glucose being retained And the concentrations found indicate that from one-fifth to nearly one-third of the water of the glomerular filtrate is reabsorbed during passage through the proximal tubule, that twice as much as these fractions represent is absorbed from the distal tubule

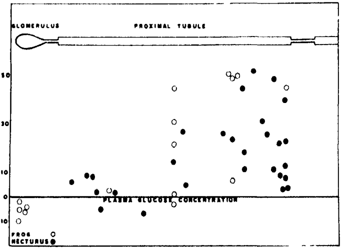

FIG 20 Chart showing percentage differences between glucose concentration of blood plasma and fluid collected from various levels of the proximal tubules of phlorhizinized Nocturi and frogs From *Amer J Physiol* 1937, **118**, 137

More objective manifestation of water reabsorption can be elicited by filling the lumen of a tubule by capsular injection with a dilute solution of phenol red (Richards and Barnwell 1927) The lumen of the tubule is at first apparent as a faintly coloured band In the course of a few minutes the colour becomes more intense and is localized in a portion of the tubule which can be identified as the distal segment The same phenomenon can be observed in a kidney the vessels of which are being perfused with oxygenated Ringer's solution the composition of which is the same, with the exception of the phenol red, as that of the fluid introduced into the tubule The composition of the fluids within and without the tubule being initially so nearly the same, the transfer of water could scarcely be due to diffusion and must therefore, we think, be ascribed to active processes within tubule cells

The observations concerning tubular function which have thus far been summarized are descriptive of conserving capacities of the kidney All or nearly all of the chloride, the glucose, the base combined as chloride and bicarbonate, and much of the water which escape from the blood in the glomeruli are restored to the blood by the activity of the cells of different parts of the tubules I wish now to show some results which illustrate the excreting capacity of the kidney

Before doing so it seems necessary to say that evidence which has accumulated within the past fifteen years has decided in the affirmative the long-debated question of the capacity of tubules to secrete I refer particularly to the work of Marshall and Grafflin (1928) and Edwards and Condorelli (1928) on urine formation in certain teleost fishes whose kidneys are not equipped with glomeruli, to perfusion experiments with frogs' kidneys, notably by Hober and his pupils at Kiel, which showed that certain dyes (Schemınzky 1929), as well as uric acid (Lueken 1932) and urea (Hober 1930), when supplied to the tubules but not to the glomeruli, are excreted in high concentration, and, most important, the experiments of Marshall and Vickers (1923) and Marshall (1931) on the excretion of phenol red by the dog's kidney in which it was proved that this dye is extracted from the blood by tubule cells and discharged from them into the tubule lumen *

We have the task of deciding whether the excretion of a substance by the kidney is the result of failure of the tubule to reabsorb it from the glomerular filtrate or whether instead of, or in addition to this, the tubule actively excretes it

The substances which were studied are urea (Walker and Hudson 1937*b*), inorganic phosphates (Walker and Hudson 1937*c*) and creatinine (Hendrix, Walker and Richards, unpublished) The results, not as unequivocal as had been hoped, are shown in fig 21 as average curves of change in concentration between glomerulus and ureter A similar curve of glucose values, constructed from the data of Walker and Hudson (1937*a*) from phlorhizinized animals is also included

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* More recently, similar evidence has been obtained that certain organic iodine compounds used in X ray urography (diodrast, 3 5 diiodo 4 pyridon N acetic acid diethanolamine and hippuran, sodium ortho iodohippurate) are in part excreted by the tubules of mammalian kidneys (Landis, Elsom, Bott and Shiels 1936, Elsom, Bott, Shiels 1936)

If, as we believe, the increase in glucose concentration is the result solely of reabsorption of water, we may say at once that the same cause is adequate to explain the rise in concentration of urea in the tubules of *Necturus* and of inorganic phosphate in those of both the frog and *Necturus*, the curves give no intimation of secretion of these substances by tubule cells

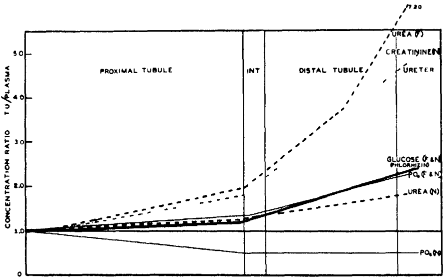

Fig 21 Average curves representing changes in concentration of urea, inorganic phosphate and creatinine in passage through the tubules of normal frogs (*F*) and Necturi (*N*) together with changes in glucose concentration in phlorhizinized animals

The marked divergence from these of the curve of urea concentration in the tubules of the frog and of that of creatinine concentration in the tubules of *Necturus* may be taken to mean—though it scarcely proves—that a fraction of urea is excreted by frogs and of creatinine by Necturi through the wall of the tubule If this is correct, the indication is that both of the chief segments of the tubule possess secretory capacity for these substances It is of interest to note that the tubules of these two amphibian species are not alike with respect to the excretion of these substances

A second curve of PO_4 concentrations has been included derived from a minority of the observations of Walker and Hudson (1937*c*) It shows that inorganic phosphate, like Na, Cl and glucose in normal animals, under some circumstances, is restored to the blood by reabsorption from the glomerular filtrate and that the selective reabsorptive process concerned operates in the proximal segment

The question now arises whether the conclusions which have emerged from these experiments with amphibian kidneys, particularly those concerning glomerular filtration and tubular reabsorption, are transferable to processes within the more highly differentiated mammalian organ The methods which I have been describing have not yet been made adaptable to mammalian study, hence the answer will be less direct I think, however, that it is none the less certain that the mammalian glomerulus is a filter and that the chief function of the mammalian tubule in the elaboration of normal urine is reabsorption

1 Study of the minute structure of the mammalian glomerulus gives no more evidence of the existence in it of a secreting structure than has been found in the Amphibia Actually it gives less, for the epithelial cells which invest the glomerular capillaries in Amphibia are more conspicuous structures than in mammals

2 The weightiest objections which in the past have been raised against glomerular filtration in the mammalian kidney have been shown to have been based upon inadequate experimentation *

3 Positive and, I think, compelling evidence has recently been obtained in the study in mammals of the circumstances of the renal excretion of substances with large molecules, particularly of inulin

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* In addition to the objections which have been discussed, two others should be mentioned Hober and Mackuth (1927) found that urine formation in the perfused frog's kidney is reversibly decreased by asphyxia (KCN or O_2 lack) They attributed this to inhibition of active secretion by the glomerulus because the arterial perfusion flow through the kidney—their criterion of *glomerular* perfusion—was not lessened They failed to recognize that many other arteries than those which supply glomeruli are reached by the arterial perfusion fluid and that these react to asphyxia by dilatation while glomerular arteries and arterioles react by constriction It seems certain, therefore, that the diminution in urine was the result of lessened glomerular pressure and flow (Beck, Kempton and Richards 1938)

Holton and Bensley (1931) have recently upheld Heidenhain's denial of the excretion of indigo carmine through the rabbit's glomerulus because, like him, they were unable to find traces of the dye in the capsular spaces in sections of kidneys excised during its excretion These failures are explained by the observation that unless the glomerular filtrate contains as much as 8–10 mg % of indigo carmine, visibly coloured residues in the capsules are not to be found in sections prepared according to their technique Only about 15% of the indigo carmine in rabbit's plasma is uncombined, hence filtrable The concentration of indigo carmine in blood plasma after its intravenous injection into rabbits diminishes very rapidly When dosage and time of excision of the kidney were chosen with reference to these facts, indigo carmine was found in the capsular spaces (Kempton, Bott and Richards 1937) These findings do not, of course, invalidate the main contention of these authors that the chief site of excretion of indigo carmine in rabbits is the tubules

From the experiments with frogs, it was argued that the openings or passages in the glomerular membrane through which the filtrate escapes are larger than the dimensions of an inulin molecule and smaller than those of the molecule of horse serum albumin, the largest molecule which was found to pass through, and that probably not through the entire glomerular surface, is that of egg albumin

Now let these three substances be injected into the circulation of a dog Inulin is excreted in the urine with remarkable rapidity and completeness In one of our experiments, 70 % of an injected dose of 14 5 g was excreted in 45 min When egg albumin was injected along with an equal amount of inulin, its excretion was somewhat slower though eventually quite as complete When horse serum albumin was injected it was not found in the urine, even in traces If now we have proof that neither inulin nor egg albumin is excreted even in part by the tubules of the mammalian kidney we should be able to say that the "mesh" of the mammalian glomerular membrane is approximately the same as that of the amphibian and that glomerular excretion is solely the result of filtration

Concerning egg albumin, there is much evidence, chiefly histological (Bayliss, Kerridge and Russell 1933), that it escapes through the glomerulus, none, so far as I am aware, that the tubules excrete it

Concerning inulin, we know that the aglomerular kidney of teleost fishes is unable to excrete it, even when injected intravenously in large doses (Richards, Westfall, and Bott 1934, Shannon 1934) The same is true of the frog's kidney when inulin is supplied only to the tubules (Richards, Bott and Westfall 1938) In dogs and rabbits in which renal arterial blood pressure has been reduced to or below the colloid osmotic pressure of the plasma proteins, thus rendering glomerular filtration impossible, injected inulin is not excreted into lumina of tubules, whereas simultaneously injected phenol red or hippuran is (Richards, Bott and Westfall 1938) In dogs and rabbits the concentration ratios, U/P, of injected inulin and creatinine are the same (Richards, Westfall and Bott 1934, 1936, Shannon 1935*a*, Kaplan and Smith 1935) This means that their rates of excretion in relation to concentration in plasma (plasma clearances) are identical In these animals, and also in man, during phlorhizin poisoning, their rates of excretion are identical with those of glucose (Shannon and Smith 1935, Shannon 1935*b*) If one is to believe that secretory processes in tubule cells participate in the excretion of these substances he must make the incredible assumption that they deal with these three widely different substances identically, whereas if filtration is the sole excretory process the rates of their removal from the plasma must inevitably be the same, provided only that the glomerular filter is permeable to all alike, as, in frogs, we have found that it is For these reasons we believe the glomeruli of higher mammals and man to be filters with nearly as sure conviction as that held concerning the amphibian glomerulus

In Cushny's development of the theory that the only processes of urine formation are those of filtration and reabsorption it was implicit that the rate of filtration could be quantitatively measured if some constituent of the glomerular filtrate were found which is not reabsorbed from the tubules The amount of such a constituent excreted in the urine per minute divided by the amount contained in 1 c c of plasma filtrate would give the total volume of glomerular filtrate formed per minute Rehberg (1926), accepting Cushny's fundamental assumptions, suggested that creatinine might be such a substance, its concentration ratio (urine/plasma) being the highest of any of the normal constituents of plasma and urine He published the results of such measurements made upon himself The validity of his suggestion rested upon the then unproved assumptions that creatinine is completely filtrable through the glomerulus, is not excreted by the tubule and is not reabsorbed from it The facts stated above justify belief that the first two of these assumptions are true with respect to inulin Its low diffusion coefficient, the magnitude of the values of its clearances and their identity with those of creatinine in dogs and rabbits indicate that little if any is reabsorbed Hence we have confidence in accepting the inulin clearance, and hence that of creatinine, in these animals as an actual measure of the glomerular filtration rate In man, creatinine clearance exceeds that of inulin by from 30 to 40% (Shannon 1934) From this it has been concluded that the tubules of the human kidney secrete a corresponding fraction of the urinary creatinine There are enough uncertainties, however, concerning the origin of urinary creatinine to justify reservations in the acceptance of this conclusion

Many determinations of inulin clearance have now been made, the majority in the laboratory of Professor Homer Smith at the New York University Medical College In rabbits (Kaplan and Smith 1935), at urine rates above 1 c c /min , it averaged about 55 c c /sq m /min , in dogs (Shannon 1935*a*) the average figure at urine flows of from 0 4 to 7 c c min was about 65 c c /sq m /min , in man the average inulin clearance was found to be about 70 c c /sq m /min For "ideal" man of body surface of 1 73 sq metres the glomerular filtration rate estimated from inulin clearance is about 120 c c /min (Smith 1937) This value is obtained whether urine is being excreted at the rate of 0 5 c c /min as a result of water deprivation or 15 c c /min because of excessive water intake, or at intermediate rates

We come at last to a conclusion substantiated by a large volume of evidence, direct and indirect, which seems to me to be of first importance in any consideration of kidney function viz that the beginning of urine formation consists in the separation from blood of a torrent of undifferentiated filtrate by a blind physical force Its volume is so great as to contain all the normal constituents of plasma which must be excreted, with the possible exception, for man, of a fraction of the creatinine, it contains also large quantities of glucose, amino acids and salts which must not be excreted In the cells of the tubules reside the capacities of restoring to the blood by finely adjusted selective processes of reabsorption those substances which the body must keep

In tubule cells also reside secretory capacities which are responsible for the excretion of most or much of some foreign substances of which phenol red and certain organic iodine compounds may be taken as typical, but in the present state of our knowledge we are not required to believe that any of the urea, uric acid, anions or fixed bases are normally excreted otherwise than by filtration through the glomerulus In the formation and elaboration of normal urine in higher animals and man the work of excretion is largely, if not wholly, performed by the heart through the medium of the kidney by the maintenance of adequate glomerular circulation The normal work of the kidney itself consists in very large part in salvaging to the organism those constituents of the glomerular filtrate whose loss would entail irretrievable damage

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FERRIER LECTURE

The localization of activity in the brain

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[Plates 15–20]

Sixty-eight years ago Fritsch and Hitzig (1870) published their first paper on the electrical excitability of the cerebral hemispheres Following the doubtful indications of an experiment on man they had tried the effect of galvanic stimulation on the exposed cortex of a dog It was the general belief that the cerebral cortex was inexcitable, but Hitzig found that a current applied to certain areas would produce characteristic movements of the head and the limbs Their paper marks the beginning of a new era in cerebral physiology Until it appeared animal experiments had failed to demonstrate any localization of function in the cerebrum, and although certain nervous disorders in man suggested it, the general opinion was that the cerebrum was too highly organized to admit of any regional differences in behaviour The new result agreed with the clinical evidence, with Broca's asphasia and Jackson's speculations on epilepsy, but it went very much further it showed, in fact, that the activities of the cerebrum were within the reach of experimental analysis

Three years later David Ferrier (1873) developed the full possibilities of the new method of research He was then a man of thirty working in the laboratory maintained at the West Riding Asylum by James Crichton Brown Many of us have seen these two eminent Victorian physicians in their old age and will be glad to keep the memory of Ferrier's slight, alert figure and Crichton Brown's dignified vitality It is a memory which links us to a classical period in the history of medicine, the period when neurology became a science

Ferrier confirmed the work of Fritsch and Hitzig, but he was able to go much further than they had done He found that the faradic current was a much more effective stimulating agent than the galvanic and with this improvement in technique was able to map out the excitable regions of the brain in much greater detail With the repeated stimuli instead of the constant current the character of the movements stood out more clearly and there was no danger of injuring the cortex by the stimulus Within ten years he had established the main facts relating to the localization of the motor centres in the cerebrum and had already seen how these facts might be used as a guide to surgical operations

The facts of cerebral localization which Ferrier established have never been seriously questioned Their value as a guide to cerebral organization has been more in dispute, for there have been times when they have seemed a clear guide to the nature of cerebral mechanisms in general and other times when they have seemed to show merely how the cerebrum is linked to the muscles Changing fashions in physiology have been partly responsible, but there have been fresh data pointing to further possibilities of analysis and fresh difficulties pointing to the folly of expecting any such analysis to explain the highly organized behaviour of man

STIMULATION OF THE HUMAN BRAIN

The position is illustrated by a consideration of recent work by electrical stimulation of the human cortex A Ferrier lecture ought in any case to refer to the valuable work in this field which is now possible to the neurosurgeon and to mention in particular the detailed maps of the excitable areas published by Foerster (1931) and this year by Penfield and Boldrey (1938) The results from man are in close agreement as far as concerns the motor centres with those obtained by Leyton and Sherrington (1917) in their classical study of the chimpanzee's brain For the sensory and other areas there is, of course, the great advantage that a man whose brain has been stimulated electrically can say what he felt and thought at the time, but although the stimulus produces sensation and movement and even the desire to move, it does not give rise to the organized thought or behaviour which is the finished product of cerebral activity Up to the present, therefore, these human results, though intensely interesting, support the view that stimulation of the cortex can reveal the receiving and executive apparatus of the cerebrum but can show very little of the processes which intervene between the incoming and the outgoing signals Thus there is no doubt that incoming signals from the eye arrive in the striate area of the occipital lobe and that outgoing signals to the arm are sent from a part of the precentral gyrus Such facts are of great practical value and they give definite information about the mechanism of the cerebrum, yet it is only the mechanism of the entrances and exits, and if we are to find out what goes on inside we must turn to the less immediate methods of clinical observation, animal training and the like

How far, then, can such direct study of the living brain lead us and how can we learn more than the anatomical map which is already so complete? This lecture will discuss some recent methods of adding to our knowledge Many of the results are difficult to assess, but at least the methods are in direct succession to those used by Ferrier, since they depend on an electro-physiological technique

EFFECTS OF REPEATED STIMULI FACILITATION

It would be a mistake, however, to turn at once to different methods, leaving the impression that electrical stimulation of the motor area gives no hint as to the nature of cerebral processes One such hint dates back to the controversy between Hitzig and Ferrier over the best form of stimulation It rests on the fact that a rapid succession of stimuli are necessary for the production of many of the typical movements To quote Ferrier "The closing or opening shock of the galvanic current causes only a sudden contraction in certain groups of muscles, but fails to call forth the definite purposive combination of muscular contractions which is the very essence of the reaction and key to its interpretation" The explanation of this superiority of the faradic current was revealed in the work of Graham Brown and Sherrington (1912) When a point in the motor area is stimulated for successive brief periods the movement evoked becomes larger and larger at each stimulation This is the phenomenon of ' primary facilitation" and it has many parallels in other parts of the nervous system, peripheral as well as central But as the response increases it is found that there is also an increase in the area on the cortex from which that particular movement can be evoked Ultimately, therefore, the whole of a large area will give only the one movement although before the stimulation several different movements might have been produced from it This is ' secondary facilitation" leading to the "deviation of the response" It is an effect peculiar to the cerebrum and shows that the motor centres have traces of the plasticity which is the main feature of cerebral organization

The changes which occur in the cortex during secondary facilitation have not yet been fully analysed It is clear that repeated stimuli can exalt the responsiveness of one particular pattern of neurones within a given region and it is probable that other patterns are correspondingly suppressed The pattern which is chosen is under the control of the experimenter in so far as it is determined by the facilitation produced by electric stimuli, but sometimes a dominant pattern is there already and it is not possible to change it It is not known why the favoured response changes from one day to the next and all that can be said is that the previous activity of the neurones is probably an important factor For this reason, if for no other, the nature of secondary facilitation is an urgent problem of cerebral physiology

There is another way of studying the effects of repeated stimulation on the cortical neurones and it may be mentioned now because it introduces the particular method with which we are mainly concerned It consists in recording not the movements of the limbs but the electrical activity induced in the cortex itself By this method it can be shown that the phenomena of facilitation are not confined to the motor area The large areas which are called silent because they do not give rise to movement are none the less capable of excitation, for each stimulus produces the characteristic potential change which is the sign of activity With a series of stimuli the action potentials become larger and larger and it can be shown that they arise from wider and wider areas with the stimulated point as centre (Adrian 1936) Thus repeated stimulation enhances the activity of any region of the cortex to which the stimuli are applied

These phenomena of facilitation have been considered because of the part which they may play in the building up of patterns of response within the cortex They become particularly important in the light of an entirely different set of data acquired within the last few years These are the outcome of the method illustrated above, that in which the cortical activity is recorded by its electrical effects

ELECTRICAL ACTIVITY OF THE CORTEX

Since the beginning of electrophysiology it has been known that the cells which can be made active by an electric current will give rise themselves to a current when they are active Such currents have long been used as an index of what is happening in a muscle or nerve, a record of the potential changes between two points showing the brief waves of activity which spread along the fibres With the recent increase in the range of electrical technique the method has become applicable to the brain as well Indeed it is now so popular that it has become very hard to separate the significant results from the insignificant

Although potential changes from the cerebral cortex had been recorded before, the most interesting point about them was not established until the work of Prawdicz-Neminski (1913, 1925) He used the string-galvanometer to register the currents from the exposed cortex in dogs and found a constant oscillation, spontaneous in so far as it persisted in the absence of sensory stimulation or of movement From this and from the character of the response to stimuli he concluded that the cortical neurones have a "special tendency towards periodic activity"

POTENTIAL WAVES IN HUMAN AND ANIMAL BRAINS

The same tendency was illustrated in a startling fashion when Berger (1929) discovered that potential changes from the human brain could be detected through the unopened skull He found that in a subject at rest, with eyes closed and attention unoccupied, a regular series of potential waves could be recorded from the surface of the head The waves recur with a frequency of about 10 per sec and constitute Berger's α rhythm There is no doubt that they arise in the cerebral cortex and express the activity of the neurones there It was thought at first that these waves came from every part of the cerebrum and showed the true unity of its behaviour In animals, however, no such uniformity of response had been observed Kornmuller (1932) soon afterwards established the fact that, under certain conditions at least, there might be as many different types of electrical activity as there were different types of structure in the cortex, each cyto-architectural area giving potential oscillations with characteristic wave forms, frequency and degree of regularity Thus the records from the brains of man and of other animals agreed in showing a persistent activity in the cortical layers, but disagreed in showing uniform activity in the one type of brain and diverse activity in the others

Further work has made it clear that there is no real contradiction between Berger's finding of the regular α rhythm over wide areas and the many observations which prove that different parts of the cerebrum may react differently The α waves in man are not produced under all conditions of activity or from all parts of the cortex As a rule they come mainly from the occipital regions and then only when visual attention is completely relaxed In certain subjects they have been found to arise in the frontal and parietal regions as well, and in other subjects they have not been found anywhere With such wide variations in origin it is clearly impossible to relate the waves to certain localized structural areas and the important point seems to be that they are characteristic not so much of a particular region as of a particular condition of the cortical neurones The condition seems to be that they should not be exposed to any disturbing influence, for in normal subjects the waves in the occipital region only appear when visual attention is not aroused This explanation involves the view that the state of the cortex may bring into prominence either the local differences in behaviour or the properties shared by neurones in general There is nothing unreasonable in this, for in animals the type of persistent electrical activity in a given cortical area may be altered out of all recognition by a change from one kind of anaesthetic to another Indeed Berger's findings on man can be repeated on animals the different areas certainly preserve their characteristic types of response under the standardized conditions used by Kornmuller, yet Ectors (1936), Remple and Gibbs (1936) and Rheinberger and Jasper (1937) have found the simple α rhythm over wide areas when the animal is in the suitable state of placid contentment

Records of the cortical potentials raise fresh hopes of analysing the working of the cerebrum for they show the constant activity in every part of it and not merely in the arrival and departure platforms In theory, therefore, they should make it possible for us to see the whole cortical organization at work In practice we are very far from this One reason is that we are still quite ignorant of the meaning of most of our records in terms of cell reactions and cell structures Another is the great variability of the results, for in no two experiments will the brain respond in exactly the same way, and indeed we could scarcely expect that it would Finally, there is the difficulty of knowing where to begin, when so many different observations can be made with so little certainty that one line of enquiry is better than another

These are reasons for not expecting a very rapid advance, but the method has already led to some general conclusions relating to the factors which modify the degree of activity in different parts of the cortex They touch on the whole problem of localization and will be worth summarizing if only to show what different aspects this problem may assume

LOCAL POTENTIAL WAVES DUE TO SENSORY STIMULATION

The electrical changes which can be most readily controlled are those produced in the receiving areas of the cortex by the stimulation of sense organs and sensory nerves The regions in which they appear correspond very closely with the sensory areas mapped out by other methods They are not a recent discovery for they were reported by Caton in 1875 as a sequel to Ferrier's work But with modern instruments they can be analysed in detail In the post-central area for instance Marshall, Woolsey and Bard (1937) find that sharply localized potential waves are evoked by light touches on various parts of the body In the auditory area (Bremer 1937) and the visual (Bartley and Bishop 1933) the distribution of activity seems to be more general, but in the latter the boundaries of the electrically active region correspond very closely with those of the striate area (cf fig 1, Plate 15)

The form of the potential waves has been studied with great care by Bartley and Bishop, their method being to stimulate the optic nerve electrically and record the response in the striate area They have found several waves, varied in magnitude by a cyclical change of excitability in the cortex, and Bartley and Heinbecker (1938) have recently found that the response in the post-central region is built up out of the same elements

These local waves may represent either the arrival of a sensory volley in the terminal dendrites of the afferent fibres or the response of the cells which are exposed to the volley Their exact origin is still uncertain for they occur in an extremely complex structure which cannot be dissected into its components Various methods of analysis have been used, e g recording by micro-electrodes (Forbes and others 1937), thermocoagulation of the outer layers of the cortex (Dusser de Barenne 1934) or local application of strychnine or novocain (de Barenne, Bartley and Bishop), and these have already narrowed the issues considerably It does not seem possible to apply the method of analysis by conducted electrotonic effects which Barron and Matthews (1938) have developed so successfully for the spinal cord, but the analysis of spinal cord potentials may help to explain those in the cortex

From the point of view of cerebral organization the most interesting point about the local electrical response is not its close dependence on the stimulus but the cyclical variations in size which Bartley and Bishop are investigating These give evidence of direct interaction between the afferent signal and the cortex and, as will appear later, it is the interaction between the local excitation and the background of spontaneous activity which is an essential feature of the cortical response Attempts to trace the effect of the local response on surrounding regions are met by various difficulties, e g those arising from the lack of a quiet background and from the use of an anaesthetic to put the brain out of action But fortunately these difficulties do not prevent the investigation of one very important consequence of the local excitation In fact the anaesthetic, by depressing the general level of spontaneous activity, brings into greater prominence the facilitation which may follow a stimulus

General level of activity in the brain

(a) *Sleep*

Before discussing the electrical records of anaesthetized brains it may be as well to consider a more familiar example of a change in the level of activity of the brain following a stimulus The example is simply that of a sleeper roused by a knock on the door If the knock is not very loud it may produce merely a slight return of tone in the muscles, a change in the breathing and perhaps a readjustment of the limbs if it is louder it will produce a sudden return to the fully integrated activity of waking life, and the change from deep sleep to waking involves an increased response in every part of the central nervous system, a return of the spinal reflexes as well as a return of consciousness

The waking of the brain is reflected in the change in the potential waves which can be recorded from it In deep sleep occasional large excursions take the place of Berger's α rhythm, in lighter sleep various new rhythms appear and there are occasional short groups of the α waves, and the α rhythm reaches its maximum regularity when the sleeper is awake but has not opened his eyes The increased activity on waking is shown in animals as well as in man, for Bremer (1936) has found it possible to lead directly from the cortex and has recorded the change from an occasional group of waves in the sleeping brain to a constant succession of them in the waking

(b) *Narcosis*

In an animal anaesthetized with one of the barbiturates the level of activity may show the same kind of alteration With dial, for instance, as in natural sleep, there are occasional periods of slight restlessness, sometimes without obvious cause and sometimes induced by sensory stimuli As in sleep these periods are associated with an increase in the electrical activity of the cortex There is, of course, the difference that with an adequate stimulus the state of natural sleep may be changed to one of waking activity which maintains itself for hours, whereas under dial the waking never proceeds beyond its initial stages The anaesthetic has produced a state in which prolonged activity is impossible, the stimuli soon lose their effectiveness and the limbs become flaccid again (fig 2, Plate 15)

Regional awakening

If electrical records are made from the brain in dial narcosis it is found that the increase in activity caused by a sensory stimulus is not only short-lived but may be confined to the limited area of the cortex in which the sensory message arrives This localized or regional awakening can be seen in records from the striate area, where the spontaneous activity takes the form of an occasional series of abrupt waves with a frequency ranging from 12 per sec in moderate to 6 per sec in deep narcosis If the eye is illuminated the signals which arrive from the optic nerve set up more rapid and irregular potential waves (fig 3, Plate 16) and there is a renewal of these when the light is turned off But the point with which we are now concerned is that the directly induced activity often leaves behind it a period in which the spontaneous activity is increased If the waves were present before the illumination, they become larger and more regular after it, and if they were absent the light may start a long series of them (fig 4, Plate 16) As a rule the effect is confined to the striate area and there is no increased activity in other regions

The same result, the increase in spontaneous activity following the arrival of a signal, can be seen in other areas than the striate Bremer has shown it in the auditory area of the cat, and fig 5 (Plate 16) gives an example from the post central region of the monkey Here the spontaneous waves are larger and more complex than in the striate area In the absence of stimulation they occur in sudden irregular outbursts at rare intervals, and, as the figure shows, *passive flexion of one of the fingers may start a* continued series lasting many seconds

Under moderate anaesthesia the increase in activity caused by a sound or a touch is confined, like that caused by a light, to the cortical area in which the signals arrive With more urgent stimuli there may be a more widespread increase, but when this occurs the local patterns of activity are preserved Thus pinching the finger may start the 10 per sec rhythm in the striate area as well as the larger and slower waves in the post central, in light anaesthesia the same effect may follow a touch or it may occur without stimulation When it follows a pinch the increase in activity is most probably due to the arrival of pain signals in the thalamus rather than of touch signals in the cortex It is unlikely, however, that the processes involved are very different

So far then, the electrical evidence has shown that there is normally a persistent activity in all parts of the cortex, that it may be greatly reduced by an anaesthetic such as dial which produces a state resembling sleep, and that it may then increase either locally or generally when a sensory message arrives Some process akin to facilitation might explain how the message wakes up the surrounding area, but we can learn more by turning to another line of evidence The same kind of regional awakening can be produced by local stimulation of the cortex as well as by an afferent message and although the whole process is more artificial it is in some ways easier to follow

ACTIVITY INDUCED BY LOCAL STIMULATION

An electrical stimulus to any part of the cortex will cause a local excitation and a local potential change not very different in form from that produced by a sensory message In the surrounding areas it produces two distinct effects, both referable to some form of facilitation but referable to different levels of activity They are (1) a direct transmission of the excited state from neurone to neurone, and (2) a general increase in the excitability, or in the tendency to continued discharge, in the neurones which are not directly excited

The first, more direct, effect is best seen in the cortex of the rabbit when repeated stimuli have produced a high degree of responsiveness (Adrian 1936) Each stimulus of the series (if the frequency is not too high) will then set up a wave of activity which travels outwards from the stimulated point into neighbouring areas (fig 6A, Plate 17) As the facilitation increases the waves become larger and spread further and further until ultimately each of them travels outwards over a circle 1 cm or more in diameter Within this area no other form of activity can occur If the increase continues, the regular series may be broken by waves starting from fresh foci within the affected region, and long after-discharges may occur when the stimulation is over (fig 6B, Plate 17) On the other hand in deep narcosis the building up of the facilitated area may be cut short by a rapid failure after the initial increase in the response

The waves produced by repeated stimulation are chiefly interesting on account of their resemblance to the waves which can be recorded from the brain during epileptiform convulsions Both in those due to disease and to convulsant drugs we find large potential waves of the same form spreading over the cortex and monopolizing its whole activity Granted the focus of persistent rhythmic excitation it is not very difficult to understand how one group of neurones after another may come to discharge in phase with the pacemaker, so that a pulse of synchronized activity spreads through the cortical neurones as it does through the heart muscle It is true that this form of activity is quite abnormal for the brain but it shows how facilitation may operate in an extreme case

The other result of local excitation must be studied in equally artificial conditions but does not involve such an abnormal degree of facilitation

It is the effect resembling that of a sensory message, the local excitation starting a train of waves of the same type as those which make up the normal persistent activity As usual it is necessary in describing the behaviour of the brain to specify both the animal and the anaesthetic In the monkey under dial it is possible to show the different patterns which can be evoked by a stimulus to different parts of the cortex, but the way in which the stimulus operates can be followed most easily in the cat's brain in fairly deep narcosis with dial or nembutal In these conditions, as Bremer has shown, the spontaneous activity takes the form of groups of waves separated by quiet intervals of 3–4 sec (fig 7, Plate 17) The waves in each group occur at a frequency of 6–11 per sec and are isolated spikes rising from a quiet base line With surface electrodes the potentials are mainly due to the superficial structures of the cortex, though similar groups can be detected in the deeper layers (fig 8, Plate 17)

When the narcosis is so deep that sensory stimulation has no effect, the groups appear with a regularity not far removed from that of respiration As the narcosis deepens the groups become shorter and the quiet intervals longer, until ultimately there may be no more than an occasional isolated wave It is at this stage that the effect of stimulation of the cortex is most obvious, for one or more shocks will then start a typical group of waves (figs 9, 10, Plate 18) In the lighter stages the effect may be obscured by the spontaneous groups, but if the interval between them is long it is easy to show that a stimulus can make a group appear earlier than it would otherwise have done In the records which illustrate this (fig 10) it will be seen that if the stimulus falls very early in the quiet interval it will either have no effect (apart from the local response) or will produce a group of only a few waves the later it falls the larger will be the response to it It seems, therefore, that after each group has occurred there is a gradual return of the conditions favourable to activity, that ultimately this will lead to a spontaneous discharge, but that a local excitation can act as a trigger which will start the discharge before the breakdown point is reached

It may be mentioned that in the rabbit the regular alternation of active and inactive periods is rarely found, but in deep narcosis where there is very little spontaneous activity a single stimulus will set up a rhythmic succession of waves The duration of the group then follows the same rule as in the cat, lasting 2 sec or more if the cortex has been at rest and only ½ sec if it has been recently stimulated to activity

The grouped discharge represents a release of activity which may spread over a fairly wide area once it has been started by a local excitation, spontaneous or induced The way in which the spread takes place can be followed by recording simultaneously with several oscillographs from a number of electrodes arranged in line on the cortex It can then be seen that the waves of activity are rarely stationary, for the region of maximum negatively on the surface usually shifts a few mm along during each wave When the group is started by an electrical stimulus the first few waves are developed at the stimulated point and may or may not move a short distance (fig 11, Plate 19), but as one wave succeeds another the starting point may shift gradually so that the activity spreads into fresh areas Thus the last wave of a group may start at a point as much as 2 cm away from the first one (fig 12, Plate 19)

The shift in origin occurs as much in groups which have developed spontaneously as in those induced by a stimulus It varies greatly in extent, but it is noteworthy that a considerable shift can occur without any marked disturbance in the regularity of the series It must, therefore, be due to fresh neurones becoming involved in the discharge and taking over the function of pace maker because they are now the most excitable elements in the system In fact the process resembles the development of the spreading waves with repeated stimulation, the difference being that without the stimuli there is nothing to fix the origin of the wave system

In addition to this spread of the activity by direct transmission there is a general facilitation in surrounding areas It is shown by the fact that fresh wave systems often appear during the active period and produce sudden irregularities in the records (fig 13, Plate 19) The new waves come from centres of activity which have been brought to the point of discharge by the general increase of excitability These waves will spread into other regions and so will carry the facilitation still further Thus a period of activity started at one point will tend to spread widely and if there are no competing pacemakers it will make the whole cortex follow its lead

This tendency towards the spread of activity in the cortex can be demonstrated in various ways For instance in either the cat or rabbit if the narcosis is deep enough to stop any spontaneous discharge a single stimulus to one point on the cortex will often initiate a period of activity over the whole surface And in the cat if spontaneous groups occur at fairly long intervals there is usually a general, though not a detailed, agreement in the periods of rest and activity in regions some way apart In lighter narcosis there is less chance of a single pacemaker dominating the cortex the intervals between the active periods are much shorter and there is less agreement between different points

Although for simplicity the cortex has been spoken of as though it was no more than a large sheet of cells and dendrites, there are in fact the elaborate association fibre systems which make short cuts from one point to another These must play a considerable part in the spread of activity and may explain the occasional appearance of two foci 1 cm or more apart discharging almost simultaneously Another factor of great importance is the connexion between cortex and thalamus, for there is abundant evidence to show that the two regions may reinforce one another even in deep anaesthesia Indeed destruction of the connecting fibres may abolish the spontaneous activity of the cortex and excitation of the cortex by strychnine may cause intense activity in the thalamus (Dusser de Barenne 1938)

ASSOCIATION OF NEURONES

Records of the spontaneous potentials illustrate not only the way in which activity may spread, but the way in which the neurones may form large or small associations under the influence of competing centres This can be seen in the sudden changes in size of the waves, e g in fig 14A where a regular series breaks down into small, rapid oscillations and in fig 14B where a number of distinct wave systems suddenly merge into one (Plate 20) Clearly if the cortex is in a uniform, undisturbed condition a single pacemaker may control a large area, but this would break up into a number of small territories when there are several competing foci in it Changes of this kind due to the fusion and dispersion of neurone aggregates are more often seen in lighter anaesthesia, particularly in preparations under ether In these there may be recurrent periods of activity some of which are made up of large, simple waves and some of very small irregular oscillations (fig 15, Plate 20) Sometimes there is a sudden change from the one to the other during the active period and it is sometimes possible to bring the independent foci into temporary unity by an electric stimulus which will make them all start fairly together (fig 16, Plate 20) On the other hand a unified wave system is sometimes broken up by the sensory stimulation of some part of the body

INHIBITION

These records illustrate some of the general principles governing cerebral organization such as the tendency to periodic discharge in the cortical neurones and the tendency for activity to spread, etc , but it must be remembered that there are some very important factors which are not shown They could scarcely be looked for in the narcotized brain where the level of activity is already very low, for they are the factors which check the tendency for the level to rise General exhaustion of available energy would be one such factor and it may be operative in the records of the grouped discharge, but inhibition is far more important In the normal working of the cerebrum the spread of activity is directed into particular channels which vary from moment to moment and this canalization must involve inhibition as much as excitation Indeed the development of any organized response would be quite impossible without some means of controlling the tendency for activity to spread in all directions But inhibition must be studied by other methods and we are now concerned only with the excitatory factors

"SPONTANEOUS" ACTIVITY

The foregoing account has emphasized the importance of sensory stimulation in raising the level of activity in the cortex But it has made use of the phrase spontaneous activity and has never defined its meaning The account would not be much the worse for the lack of precise definitions yet the word "spontaneous" is a frequent cause of misunderstandings These are worth avoiding and a discussion will also serve to show that the neurones in the cortex do not differ fundamentally from neurones elsewhere

The confusion which may arise when we contrast induced and spontaneous activity is illustrated by considering the rhythmic movements of breathing The periodic inflation of the chest (in the mammal) is normally timed by periodic signals from the sense organs in the lungs, but if these are eliminated by cutting the vagi the movements still occur though at longer intervals It is found, however, that there is now no group of sense organs with the specific function of starting or stopping each inflation of the chest Apparently the discharge of motor impulses starts when the respiratory centre has recovered to the point of spontaneous overflow and stops when the available energy is used up

This discharge of the deafferented respiratory centre can be described as spontaneous, for there is no periodic stimulus to start each movement But a constant stream of afferent impulses still enters the spinal cord by every nerve root, and although none of the sensory messages has a direct effect on the respiratory centre their presence in the background may be a factor in maintaining its activity It is true that respiration continues in very deep anaesthesia when much of this background is probably cut off, on the other hand the facilitating effect can be seen very clearly in certain conditions in which the activity of the centre is far below normal Thus in a rabbit under very deep dial anaesthesia the breathing may slow down and finally cease altogether Pinching the foot will then restore the movements for a short time, although the anaesthesia is deep enough to prevent any effect on the blood pressure or the tone of the limbs By some process of irradiation the afferent impulses have raised the excitability of the centre to the level at which discharges occur In the same way Barcroft and Barron (1936) find that in the foetal sheep a stimulus like a tap on the head will start a series of respiratory movements some time before these movements occur regularly

Another example recalls two names particularly appropriate to this special occasion In his Hughlings Jackson lecture in 1931 Sir Charles Sherrington discussed the scratch reflex, that other rhythmic movement which in his hands has taught us so many principles of spinal integration In this, as in respiration, a complex pattern of movement is repeated periodically and periodic afferent signals are not needed to determine the rhythm But here the activity as a whole is much more clearly dependent on a particular afferent inflow, though the inflow need not be rhythmic scratching, in fact, is certainly a reflex rather than a spontaneous effect Midway between scratching and respiration come the rhythmic movements of locomotion in the leech, as reported in a recent paper by Gray and others (1938) These movements are much more persistent than the scratch reflex but if they are to continue there must be some inflow of sensory impulses from the stretch receptors There is no need, however, for the inflow to be periodic a steady stream will raise the level of excitation in the central nervous system and the periodic discharge will then occur In the intact animal it would, no doubt, be modulated by a periodic sensory inflow just as the respiratory discharge is modulated when the vagi are intact

It would certainly be possible to multiply these examples without limit and to find every shade of spontaneity in the central nervous system, ranging from the electrical rhythms which continue in isolated parts of the brain to reflexes which are no more than a direct response to an afferent signal The same wide range of behaviour could, of course, be found in excitable tissues outside the central nervous system But in the brain it is important to recognize that afferent impulses may produce both the facilitating effect on spontaneous patterns and a direct excitation of certain elements, and to recognize also that within the cortex there may be the same wide variation in the tendency to spontaneous discharge as there is elsewhere

The conclusion, therefore, is that the activity set up in a given region of the brain by an afferent message will be determined by two distinct factors One is the actual content of the message, the concentration of impulses from moment to moment, which will tend to produce a corresponding ebb and flow of excitation the other is the tendency of the region to respond, when excited, according to some pattern of its own determined by its structure and, we may suppose, by its history Thus the brain will receive the message and will interpret it in its own way

These considerations lead us to study not so much the detailed anatomical localization of activity in the brain as the general reactions of the cerebral apparatus We have to enquire what physical and chemical changes have occurred in an area which has been roused by afferent impulses, what happens in the neurones to make them discharge periodically and particularly how the pattern of response is established and modified These problems seem at present to be nearer solution than any problem of detailed behaviour could be For the chief difficulty in cerebral physiology is the enormous amount of detail which it must ultimately include, and the chief problem is to decide the best line of approach to such complexity

The new methods of electrical investigation have added more detail, but have already shown some of the lines on which it may be analysed From time to time no doubt the task of analysing these reactions will seem hopeless in the face of some new difficulty brought forward by those who are mindful of the organic unity of the brain But equally from time to time we shall find out more about the mechanisms which are undoubtedly present in it and out of which its behaviour must be built up and it is difficult not to believe with Ferrier that the analysis is worth attempting

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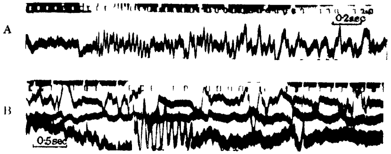

Fig. 1. Electrical response of the striate area.

A. Monkey under ether anaesthesia. Illumination of the eye marked by signal (white) above. The waves start with a frequency of 15 per sec.

B. Monkey under dial. Simultaneous oscillograph records from three pairs of electrodes: the lowest tracing is from the striate area and shows the outburst of waves when the eye is illuminated; the middle tracing is from a region 1 mm. in front of the Affenspalte and the upper is from the post central area. The increased activity is confined to the striate area.

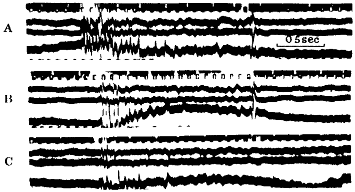

Fig. 2. Fatigue of cortical response with repeated afferent stimulation. Monkey under dial anaesthesia (deep). Response from the left striate area to three periods of illumination of the eye: interval of 15 sec. between A and B and of 10 sec. between B and C. The three oscillographs record from different points of the striate area. With lighter anaesthesia the diminution in successive responses would be much less.

ELECTRICAL RESPONSE OF THE STRIATE AREA ON ILLUMINATION
OF THE EYE

(*Facing p.* 448)

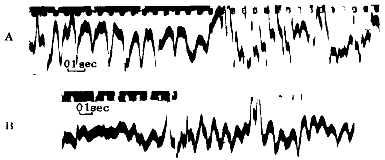

FIG 3 Response from the striate area on illuminating the eye

A Monkey under dial Potential waves at 11 12 per sec rising to 30 per sec on illumination

B Another animal Illuminating the eye starts waves at 11 per sec

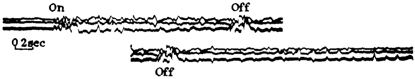

FIG 4 Response from the striate area Monkey under dial (deep) Illuminating the eye sets up an irregular discharge when the light is turned off a regular rhythm at 6 per sec is left after the 'off' discharge The two strips are from a continuous record and overlap one another

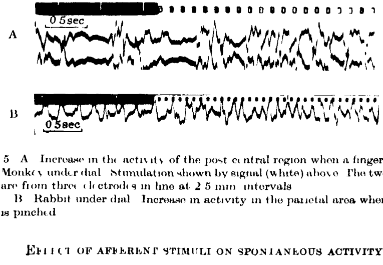

FIG 5 A Increase in the activity of the post central region when a finger is flexed Monkey under dial Stimulation shown by signal (white) above The two tracings are from three electrodes in line at 2 5 mm intervals

B Rabbit under dial Increase in activity in the parietal area when the foot is pinched

EFFECT OF AFFERENT STIMULI ON SPONTANEOUS ACTIVITY

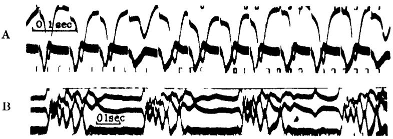

FIG 6 A Potential waves produced by direct stimulation of the rabbit s cortex Lower tracing from electrodes 1 and 3 5 mm from the stimulated point Upper from electrodes at 5 and 7 5 mm The waves travel outwards and affect first the lower and then the upper electrodes

B After discharge of the cat s cortex following repeated stimulation

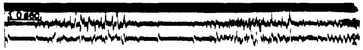

FIG 7 Spontaneous wave groups in the cat s cortex under moderately deep dial anaesthesia Discharge recorded from two regions 7 5 mm apart The groups recur every 2 3 sec

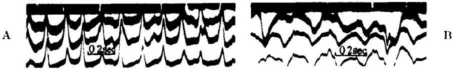

FIG 8 A Spontaneous waves from the cat s cortex recorded from three points in line at 2 5 mm intervals During the wave all the points become negative to the average potential of the brain (shown by upward movement)

B After the surface of the cortex has been cooled, positive waves (movement downwards) can be detected with higher amplification These are probably from the deeper cell layers

Cat under dial (deep) Wave frequency 6 per sec

EFFECT OF AFFERENT STIMULI ON SPONTANEOUS ACTIVITY

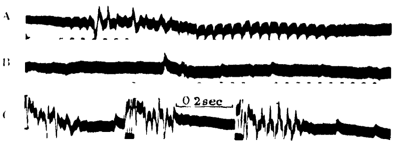

FIG. 9. Cat under deep dial anaesthesia.

A. Spontaneous group of waves at 6 per sec.

B. Deeper anaesthesia. Activity reduced to occasional waves.

C. Same state as B. Wave groups set up by short periods of stimulation. Stimuli marked at the foot of the record.

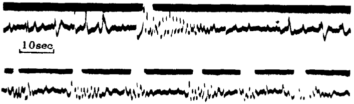

FIG. 10. Groups set up by a single stimulus showing greater response after longer period of inactivity. Signal (white mark above) gives approximate moment of stimulation with single condenser shock.

WAVE GROUPS PRODUCED BY DIRECT STIMULATION OF THE CORTEX

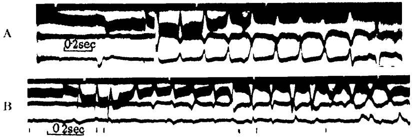

FIG. 11. Records from three pairs of electrodes in line on the cortex to show the distribution of the potential change during each wave.

A. Cat under dial (deep). Group started by single condenser shock. The potential distribution for each wave does not change appreciably until the end of the record. The stimulus is applied close to the pair of electrodes giving the top tracing and all the waves but the last are developed in this region. Electrodes at 0 and 2·5 mm., 2·5 and 5, 5 and 7·5 mm. from stimulated point.

B. Another animal. Group set up by three stimuli. After these the first five waves start from the stimulated region but the focus then shifts and the potential distribution is altered. Electrodes at 0 and 2·5 mm., 5 and 7·5 mm., 10 and 12·5 mm.

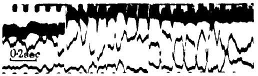

FIG. 12. Record showing a grouped discharge due to a single stimulus with a progressive shift in the point of origin of the waves. Electrodes at 1 and 3·5 mm., 6 and 8·5 mm., 11 and 13·5 mm. from stimulated point.

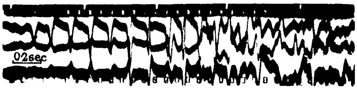

FIG. 13. Cat under dial. Grouped discharge developing spontaneously with sudden disturbance of rhythm by fresh wave systems. Electrodes at 0 and 2·5 mm., 2·5 and 5, 5 and 7·5 mm.

EVOLUTION OF THE GROUPED DISCHARGE

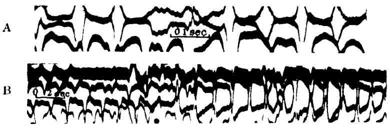

Fig 14 A Spontaneous wave group (cat under dial) In the middle of the record small rapid oscillations take the place of the large waves

B Record from four pairs of electrodes in line At the beginning each pair shows a different wave system half way through the record a single wave system becomes dominant Electrodes at 0 and 2·5 5 and 7·5 10 and 12·5 15 and 17·5 mm

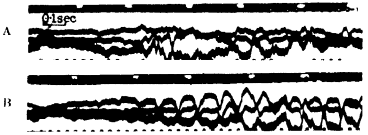

Fig 15 Cat Isolated cortex preparation (Bremer) under ether Wave groups occur every 2–3 sec and are sometimes composed of large regular waves (as in B) and sometimes of small rapid oscillations as in A

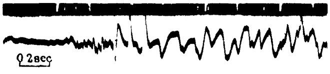

Fig 16 Cat Isolated cortex preparation under ether Periodic discharges occur as in fig 15 and the discharge can be changed from the small wave to the large wave type by direct stimulation of the cortex

CHANGES IN SIZE AND REGULARITY OF WAVES DUE TO THE FORMATION OF LARGE OR SMALL ASSOCIATIONS OF NEURONES

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Visual pathways concerned in gonadal stimulation in ferrets

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[Plates 21, 22]

Anoestrous ferrets become reproductively active in winter if the number of hours of light to which they are exposed daily is prolonged by artificial illumination (Bissonnette 1932) This response, which has also been demonstrated in wild mice and various species of birds, cannot be elicited after hypophysectomy (Hill and Parkes 1933), while it is much impaired after division of the optic nerves (Bissonnette 1935*a*, *b*, 1936) It is therefore concluded by most workers that activation of the gonads in such experiments is primarily dependent on retinal stimulation (Marshall and Bowden 1934), and on the effects which such stimulation has upon the pituitary

The present paper is a record of a series of experiments which were designed to discover possible nervous pathways through which retinal stimulation may affect the pituitary

MATERIAL AND METHODS

The experiments were performed during the non-breeding seasons October to April in 1936–7 and 1937–8 In general the aim of the experiments was to discover whether or not anoestrous ferrets whose visual pathways have been interrupted at various levels come into heat if placed under the influence of additional daily illumination As controls normal animals and animals whose optic nerves had been divided were exposed to identical conditions of illumination in both experimental seasons Normal control animals were also kept under ordinary laboratory conditions

The animals used were adult female ferrets of the polecat variety They were obtained from ferret dealers in August and September, and were kept under normal laboratory conditions until they were operated upon in November or December In all cases the vulva at the time of operation was in the typical anoestrous condition

After operation the animals were transferred to a room arranged for exposure to prolonged lighting Each was placed in a separate cage with a wire-mesh front, the cages being arranged in three rows of four, one above the other A frame carrying four 100 W bulbs was fixed at a distance of 2 ft in front of the racks, in such a way that no shadows were cast into the cages The lighting was controlled by an automatic time switch, and was turned on from 4 30 p m to 11 p m daily

After 10 days' exposure to light the vulva of each animal was examined twice a week The animals were mated with males, which had also been exposed to extra illumination, as soon as they were seen to be in full heat, and were killed by means of deep anaesthesia 3 days later The ovaries were sectioned serially, stained with Ehrlich's haematoxylin and eosin and examined in order to determine whether or not luteinization had taken place

OPERATIVE TECHNIQUE

The operations were carried out under strictly aseptic conditions In the case of the intracranial lesions, nembutal anaesthesia was employed For exposure of the visual cortex and the mid-brain, a skin incision was made in the median plane along the sagittal crest and extending for a little way down the back of the neck The large temporal muscles were exposed, and their attachments to the sagittal and lambdoidal crests were incised with the point of the scalpel The origin of the muscle was separated from the temporal fossa with the aid of a periosteal elevator, and the whole of the skull roof was thus laid bare An opening was made to one side of the mid-line with a cranial burr, and the surrounding bone was then removed piecemeal with bone forceps until an adequate exposure had been made The small amount of venous haemorrhage which is usually encountered near the lambdoid crest, and at the sinus canal laterally, was arrested by the application of bone wax The superior longitudinal sinus was then picked up with forceps and coagulated with the endothermy current, and the dura reflected so as to expose the whole of the caudal half of the cerebral hemispheres The occipital lobes were resected with the aid of the endothermy apparatus In the case of the mid brain experiments, the tectum was brought to view by further lifting up the margin of the cut hemispheres until the contour of the superior colliculi could be clearly seen The colliculi were then undercut from the anterior and lateral aspects with a fine ophthalmic knife

Bilateral section of the optic tracts was accomplished by the exposure, on either side and through a relatively small trephine opening, of the pseudosylvian sulcus and the posterior ectosylvian gyrus Following on preliminary observations which had been made on the cerebral topography of the ferret, a fine ophthalmic knife was entered transversely, immediately behind the middle of the pseudosylvian sulcus for a depth of 7 mm (see fig 1) This brings the point of the knife immediately in front of the anterior margin of the optic tract at the level of the ventral pole of the lateral geniculate body The knife was then inclined so that its point cut backwards horizontally, with the purpose of severing the optic tract immediately ventral to its termination in the lateral geniculate body and the superior colliculus

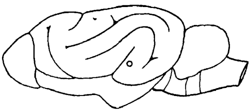

FIG 1 Lateral view of brain of a ferret showing the point at which the cystotome is entered in order to reach the optic tract × 1 6

All the operated ferrets made a rapid recovery, regaining their normal activity within a day or two

The optic nerves were divided, under ether anaesthesia, by incising the conjunctiva and rotating the eyeball forwards to allow section of the nerve in a retrobulbar position without injury to the other connexions of the eyeball

EXPERIMENTAL RESULTS

(a) *Control animals not exposed to artificial illumination*

The five unoperated control animals kept under normal laboratory conditions came into heat in the period between 4 April and 15 June

(b) *Control animals exposed to artificial illumination*

Five unoperated control animals (18, 19, 24, 52, 53) which were exposed to artificial illumination under the same conditions as the experimental animals, came into full heat after periods varying between 50 and 74 days (between 14 January and 15 February) Four of these five animals were mated, and luteinization occurred in the ovaries of all

(c) *Division of the optic nerves exposure to artificial illumination*

One (25) of the four animals whose optic nerves had been divided did not come into heat as a result of additional illumination during the winter of 1936–7 From the spring of 1937 until its autopsy in the summer of 1938 this animal was kept under normal laboratory conditions, but at no time during this period was there any sign of vulval swelling

Another of these animals (41) failed to show any vulval swelling, in spite of experimental illumination, during the 182 days following the operation The third (42) showed only slight swelling by the 174th day, when it was autopsied The fourth (16) alone came into full heat as a result of the additional lighting, but only after a prolonged period of 142 days It was successfully mated but luteinization did not occur The ovaries were small, there were no rupture points, and only the beginnings of anovular luteinization could be recognized in some three follicles

(d) *Removal of the visual cortex exposure to artificial illumination*

The operation was performed on one animal, 23 The occipital lobes of the cerebral hemispheres were both removed with the intention of eliminating visual cortical functions The extent of the area striata was judged by reference to the lateral sulcus, which has been shown in other carnivora to bear a close topographical relation to the area on the lateral surface of the hemisphere Inspection of the brain after death showed that the cortical lesion extended as far forwards along the dorsal border of the hemisphere as a point 3 5 mm behind the crucial sulcus, and down along the caudal border of the hemisphere as far as the level of the upper end of the pseudosylvian sulcus The lesion was slightly more extensive on the left

TABLE I THE ONSET OF HEAT IN EXPERIMENTAL AND CONTROL FERRETS

Nature of operation	Animal (F)	Date of operation	Date exposed to artificial illumination	First vulval changes	Full vulval swelling		Date of mating	Luteinization
					Date	Days after commencement of illumination		
Normal control, no extra illumination	28	—	—	—	4 iv 37	—	Not mated	—
	29	—	—	—	15 vi 37	—	Not mated	—
	30	—	—	—	3 v 37	—	Not mated	—
	57	—	—	5 iii 38	28 iii 38	—	Not mated	—
	58	—	—	28 iii 38	26 iv 38	—	Not mated	—
Normal control, illuminated	18	—	3 xii 36	25 i 37	15 ii 37	74	Not mated	—
	19	—	3 xii 36	18 i 37	5 ii 37	64	5 ii 37	Yes
	24	—	17 xii 36	12 i 37	5 ii 37	50	5 ii 37	Yes
	52	—	24 xi 37	24 xii 37	14 i 38	51	18 i 38	Yes
	53	—	24 xi 37	24 xii 37	14 i 38	51	14 i 38	Yes
Optic nerves divided	16	26 xi 36	3 xii 36	8 iii 37	24 iv 37	142	24 iv 37	No
	25	18 xii 36	19 xii 36	—	—	—	Not mated*	—
	41	16 xi 37	24 xi 37	—	—	—	Not mated†	—
	42	16 xi 37	24 xi 37	10 v 38	—	—	Not mated‡	—
Visual cortex removed	23	10 xii 36	11 xii 36	22 i 37	11 ii 37	62	11 ii 37	Yes
Visual cortex and superior colliculi removed	14	25 xi 36	3 xii 36	18 ii 37	8 iii 37	95	8 iii 37	Yes
	15	26 xi 36	3 xii 36	11 ii 37	25 ii 37	84	25 ii 37	Yes
Bilateral section of optic tracts	50	10 xii 37	13 xii 37	18 i 38	3 ii 38	52	4 ii 38	—§
	39	15 xi 37	24 xi 37	26 i 38	8 ii 38	76	8 ii 38	Yes
Partial section of optic tracts	20	4 xii 36	7 xii 36	1 ii 37	22 ii 37	77	22 ii 37	Yes
	22	9 xii 36	11 xii 36	28 i 37	11 ii 37	62	11 ii 37	Yes
	37	13 xi 37	24 xi 37	24 xii 37	21 i 38	58	21 i 38	Yes
	38	13 xi 37	21 xi 37	24 xii 37	21 i 38	58	24 i 38	Yes
	49	7 xii 37	8 xii 37	18 i 38	3 ii 38	57	3 ii 38	Yes

* Animal did not come into heat at any time during 515 days after division of her optic nerves

† No vulval swelling during 182 days after operation

‡ Incomplete vulval swelling by 174th day

§ Ovaries not sectioned

side (fig 2) On the medial surface of the hemisphere it reached down to the splenium of the corpus callosum, extending over and involving the intercalary sulcus On both sides the ventricular cavity was opened, exposing the alveus

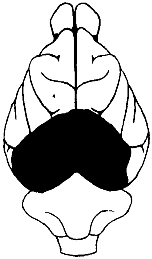

FIG 2 Dorsal view of brain of ferret 23, indicating the extent of the occipital cortex which had been removed × 1 6

Serial transverse sections were made through the thalamus and stained with methylene blue in order to ascertain the degree of cell degeneration in the lateral geniculate body and hence to assess the damage to the visual cortex It is known, on the basis of experimental work with other mammals, that the cells of the dorsal nucleus of the lateral geniculate body undergo complete degeneration after removal of the visual cortex of the same side The lateral geniculate bodies in this experiment show almost complete cell degeneration on both sides, but in the rostral part of their extent, especially on the right side, a number of normal cells remain intact It may be inferred, therefore, that the visual cortex was almost entirely ablated by the lesion, but that a minute, and perhaps insignificant, proportion of this cortical area still remained in connexion with the lateral geniculate body Serial sections were taken through the cerebral hemispheres in order to determine with certainty whether any of the visual cortex had been left intact, but the cytological features of the area striata in the ferret were found to be insufficiently distinct to allow of its positive identification and demarcation

There is considerable cellular atrophy in the pars posterior of the lateral nucleus, which lies immediately adjacent to the dorso-medial border of the lateral geniculate body, and which is known to represent a part of the pulvinar of higher mammals

This animal came into heat after 62 days' exposure to additional light Luteinization resulted from its mating

(e) *Removal of visual cortex and destruction of superior colliculi exposure to artificial illumination*

This operation was carried out on two animals, 14 and 15

Ferret 14

A lesion was made involving the superior colliculi after removal of the occipital lobes of the cerebral hemispheres Macroscopic inspection of the brain after death showed that the superior colliculi had been completely destroyed, and serial sections confirmed that all traces of them had disappeared On the left side, the lesion had also involved the dorsal half of the lateral geniculate body and had destroyed the pretectal nucleus, the large-celled nucleus of the optic tract and the greater part of the lateral nucleus of the thalamus Caudally it had extended into the ventral nucleus of the thalamus, and the rostral end of the medial geniculate body On the right side, these elements of the thalamus and the pretectal region were left intact The anterior margins of both inferior colliculi were slightly injured, and there was also a very slight involvement of the superior vermis of the cerebellum The sections show a considerable dilatation of the cerebral aqueduct The posterior accessory optic tract could not be identified on either side in the serial sections Except for a few scattered cells at their rostral extremity, the lateral geniculate bodies had undergone complete cell degeneration

Ferret 15

A lesion was made involving the superior colliculi after removal of the occipital lobes of the cortex In this, as in ferret 14, the cortical lesion was almost identical in its extent with that of ferret 23 An examination of serial sections, stained by Loyez's method and counter-stained with neutral red, shows that both superior colliculi are completely detached Their remains have almost entirely disappeared, except for small fragments of the right colliculus which, however, are wholly detached from the rest of the mid-brain (fig 3, Plate 21) The lesion, moreover, is practically confined to the superior colliculi proper, without involving the immediately adjacent structures such as the large-celled nucleus of the optic tract and the pretectal nucleus Optic tract fibres can be seen in the sections entering these nuclei on both sides without interruption The rostral margin of the inferior colliculus on the left side has been slightly injured by the lesion, and the cerebral aqueduct shows a moderate degree of dilatation The lateral geniculate bodies are intact, but except for a few scattered cells at their rostral extremity they show complete cell degeneration as the result of the removal of the occipital lobes of the cerebral hemispheres The posterior accessory optic tracts appear quite normal, the posterior commissure is almost entirely unaffected, and the oculomotor nucleus is likewise intact

These two animals came into heat after 95 and 84 days of additional illumination respectively, and the ovaries of both luteinized as a result of mating

(f) Bilateral section of optic tracts exposure to artificial illumination

The operation was accomplished in two animals only, 50 and 39

Ferret 50

After complete recovery from the operation, the animal appeared to be totally blind, showing no response at all to light or to moving objects

Autopsy 2 May 1938 Macroscopically the optic tracts appeared to be completely interrupted at the level of the ventral border of the lateral geniculate body The thalamus and mid brain were serially sectioned, alternate slides being stained by Loyez's haematoxylin with neutral red counter-staining, and with Bodian's silver method Examination of the serial sections showed the following details

Left side The lesion is a complete section of the optic tract immediately dorsal to the cerebral peduncle, and extending for a depth of about 2 5 mm into the subthalamus (*a*, fig 4, Plate 21) Not only does it interrupt the fibres of the optic tract passing to the dorsal nucleus of the lateral geniculate body and the mid-brain, but it also destroys most, if not all, of the ventral nucleus of the lateral geniculate body Anteriorly the lesion extends into the internal capsule and posteriorly it involves the lateral part of the cerebral peduncle

Right side On this side, the lesion is situated at a somewhat higher level Anteriorly it involves the lateral nucleus of the thalamus and the internal capsule, and at its posterior extremity it extends into the medial geniculate body Throughout the greater part of its extent, it interrupts the dorsolateral part of the optic tract, and has also led to a destruction of the dorsal nucleus of the lateral geniculate body (*b*, fig 4, Plate 21) Careful examination of the serial sections makes it evident that the whole length of the dorsal nucleus has been involved by the lesion A small strip of its ventral margin persists, and in odd sections an isolated cell may occasionally be seen in this region

In summary, it appears that the lesions in this animal have interrupted all visual impulses passing to the dorsal nucleus of the lateral geniculate body, the visual cortex and the mid brain, with the possible exception that the occasional cells seen in the lateral geniculate body of the right side might have been able to transmit visual impulses to the striate cortex (see below)

Ferret 39

After complete recovery from the operation, the animal was apparently totally blind

Autopsy 10 January 1938 Macroscopically, the left optic tract was seen to be completely severed at the ventral margin of the lateral geniculate body, while on the right side the lesion had involved the tract at a more dorsal level The thalamus and mid-brain were serially sectioned and stained with Bodian's silver technique An examination of the sections confirmed the conclusion drawn from the macroscopic appearance

Left side The lesion cuts completely through the optic tract at the upper border of the cerebral peduncle, penetrating into the thalamus for about 2 mm At its anterior extremity it involves the internal capsule to a slight extent While the dorsal nucleus of the lateral geniculate body is completely cut off from the optic tract a portion of the ventral nucleus remains intact and some undegenerated fibres of the optic tract can be seen apparently terminating in it

Right side Here the lesion is placed too dorsally It involves the lateral nucleus of the thalamus at its anterior extremity, and throughout the greater part of its extent it cuts through the whole thickness of the optic tract at the level of the upper border of the dorsal nucleus of the lateral geniculate body, severing all the mid brain connexions of the tract The lesion has led to a secondary degeneration of many of the cells in the anterior part of the dorsal nucleus of the lateral geniculate body, but a considerable portion of this element appears to be intact

In summary, all visual impulses to the mid-brain on both sides have been interrupted by the lesions, as well as all those passing to the dorsal nucleus of the lateral geniculate body and the visual cortex on the left side On the right side a pathway still exists through which some visual impulses might be transmitted through the lateral geniculate body to the cortex

These two animals came into full oestrus 52 and 76 days respectively after the beginning of their daily exposure to artificial illumination, and both were successfully mated The ovaries of one were serially sectioned and showed extensive luteinization

(g) *Partial division of optic tracts exposure to artificial illumination*

An attempt to divide the optic tracts as in the two experiments just described (50, 39) was only partially successful in five other animals The experiments are of interest, however, in so far as most visual impulses were interrupted by the lesions

Ferret 20

The lesion was complete on the right side, and was situated at the ventral margin of the lateral geniculate body On the left side the lesion was found to lie immediately in front of the optic tract at the same level To some extent it undercuts the anterior margin of the tract, but the latter appears macroscopically to be quite normal Serial sections show that the right optic tract is completely severed, while the left tract is only slightly injured at its rostral margin, so that a few of the fibres passing to the lateral geniculate body are interrupted The lesion can be seen on the left side to undercut the optic tract, penetrating backwards into the ventral nucleus of the lateral geniculate body, most of which is destroyed, and also involving the lateral part of the cerebral peduncle The posterior accessory optic tracts of both sides are not recognizable in the sections

In this experiment, therefore, visual impulses have been cut off on the right side from the whole of the lateral geniculate body, the visual cortex and the mid brain, while on the left side a small proportion of the retinal fibres to the dorsal nucleus of the lateral geniculate body and most of those which may terminate in the ventral nucleus have been interrupted

This animal came into full heat after 77 days' exposure to artificial illumination Luteinization occurred after mating

Ferret 22

A bilateral lesion of the optic tract was made Surface inspection of the brain after death showed that on the right side the tract had been completely transected at the ventral border of the medial geniculate body On the left side the transection was incomplete, a small strand of fibres passing to the superior colliculus at the caudal border of the optic tract being left intact (fig 5, Plate 22) These observations were confirmed by a study of serial sections through the thalamus and mid-brain, stained with Loyez's iron haematoxylin and neutral red These sections show that the complete lesion on the right side extends into the thalamus to a depth of 3 mm at its anterior end, involving here the ventral border of the ventral nucleus of the thalamus (fig 6, Plate 22) On the left side, the lesion penetrates about 2 mm into the thalamus On this side the optic tract extends over 125 sections In the most caudal 24 of these sections the continuity of the tract has not been broken, the lesion here undercutting the tract without severing it The sections show that the retinal connexions of the lateral geniculate body on the left side have probably been completely interrupted The few fibres that remain (comprising about one fifth of the width of the whole optic tract) appear to terminate in the mid brain only

In this experiment, therefore, visual impulses have been completely cut off from the lateral geniculate bodies and the visual cortex of both sides The mid-brain connexions have also been entirely interrupted except for a fine strand passing to the superior colliculus and pretectal region on the left side

This animal was successfully mated, luteinization resulting, after 62 days' exposure to artificial illumination

Ferret 37

Visual tests applied after the operation showed that the animal was apparently blind in the right eye, but retained some vision in the left eye

Autopsy 24 January 1938 Macroscopically the left optic tract was seen to be completely severed The right tract was apparently intact so far as the fibres to the lateral geniculate body were concerned, but the lesion evidently had interrupted most of the fibres passing to the mid-brain on this side

This animal was mated, and luteinization occurred in its ovaries as a result, 58 days after its first exposure to artificial illumination

Ferret 38

The animal was apparently blind in the right eye after the operation, but retained some vision in the left eye

Autopsy 27 January 1938 Macroscopically there was seen to be a complete lesion of the optic tract on the left side On the right side the lesion had cut through the caudal half only of the optic tract Probably all the fibres going to the lateral geniculate body on this side are intact though most of the fibres passing to the mid-brain must have been interrupted

This animal came into full heat after 58 days of artificial illumination Luteinization of the ovaries occurred after mating

Ferret 49

Visual tests showed post-operative retention of some vision in both eyes

Autopsy 5 January 1938 Macroscopically the lesion was found to be incomplete on both sides

This animal was successfully mated after 57 days' exposure to artificial illumination Luteinization occurred

DISCUSSION

Earlier observations on the influence of reduced illumination on gonadal function in ferrets provided somewhat ambiguous results Thus Hill and Parkes (1934) found that four of five female ferrets kept in total darkness for 23½ hr each day from the end of January onwards came into heat at the usual time, whereas Marshall and Bowden (1934) observed that only four of seven ferrets "subjected to incomplete darkness from 21 April to 23 July" came into full heat at the proper time These four animals were beginning to show signs of vulval swelling when they were put into the dark, while the three which did not respond were anoestrous at the beginning of the experimental period Bissonnette (1935*a*, *b*), in considering these findings, presents further data of a like kind, and concludes that the facts in general indicate that a reduction in either the intensity or duration of lighting will retard the onset of oestrous activity in anoestrous ferrets, or lead to sexual regression in ferrets that are already oestrous Observations on the reactions of ferrets blinded from 'natural" causes, and of ferrets whose optic nerves have been divided, clearly support the first of those two conclusions Thus Marshall and Bowden (1934, 1936) report that a ferret blinded by cataract failed to come on heat at any time between 23 December 1932 and 10 July 1935, in spite of successive periods of artificial illumination A similar observation is reported by Bissonnette (1935*a*, *b*, 1936), who also found that ferrets whose optic nerves are divided are very slow in responding to light treatment Our own observations are not only in keeping with this finding, but they also suggest that the gonads of ferrets blinded by division of the optic nerves may never become activated as a result of either natural or artificial changes in lighting

These observations suggest clearly that the primary receptor for the gonadal response is the retina Before, however, considering the possible pathways by which the visual impulses reach the anterior lobe of the pituitary gland, it is necessary to enquire whether the absence of gonadal stimulation, after division of the optic nerves is merely a secondary effect either of general nutritional insufficiency or of a diminution in general motor activity and not a primary result of the loss of specific visual impulses Neither of these possibilities, however seems likely In the first place, no differences were noted in the degree of activity displayed by the various ferrets used in our experiments Like normal ferrets, they spent most of the day curled up in sleep In the second place, all the animals ate the same quantity of food, and none showed any nutritional disturbance The possibility that blind ferrets would be unable to find their food is in any case slender, since these animals presumably depend more on olfactory than on visual receptors in their feeding activities Finally, if it be argued that the failure of the gonads to become active after illumination is a secondary effect of changes in general activity and nutrition, it becomes necessary to explain why animals which are cortically blind should behave differently in this respect from ferrets which are blind because of division of their optic nerves There is no reason to suppose that either the capacity to find food, or the impulse to move about, is different in these two classes of animal Consequently it is reasonable to seek an explanation for their different gonadal reactions to light stimulation in the different qualities of their blindness, and to take as a basis for such explanation the view that the primary process in the chain of events which leads to gonadal activity is

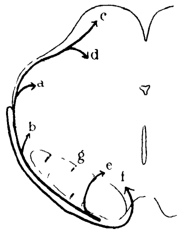

FIG 7 Diagram representing a schematic transverse section through the mid brain and thalamus, showing the possible terminal stations of fibres of the optic tract *a* = lateral geniculate body (dorsal nucleus), *b* = lateral geniculate body (ventral nucleus), *c* = superior colliculus *d* = pretectal area *e* = anterior accessory optic tract, *f* = posterior accessory optic tract *g* = subthalamus

retinal stimulation, and that the intermediate step is activation of the anterior lobe of the pituitary

In considering the afferent paths through which visual stimuli may lead to activation of the pituitary, all the terminal stations of the optic tract fibres need to be examined

It is well known that in mammals most of the fibres of the optic tract end in the dorsal nucleus of the lateral geniculate body (which forms the main part of the lateral geniculate body in higher mammals) and in the mid-brain In regard to the latter, the optic fibres end mainly in the stratum opticum of the superior colliculus An appreciable number, however, terminate in the pretectal area which lies subjacent to the margin of the superior colliculus It has been shown recently (Magoun and Ranson 1935) that this pretectal connexion provides the pathway for the pupilloconstrictor response Other possible terminations of optic tract fibres which require consideration are the ventral nucleus of the lateral geniculate body and the accessory optic tracts

The ventral nucleus of the lateral geniculate body is to be regarded morphologically as a lateral extension of the subthalamic region of the diencephalon Although it has been accepted for many years (since Cajal's original observations, 1911) that retinal fibres or their collaterals end in relation to the cells of the ventral nucleus this has recently been called into question (Barris, Ingram and Ranson 1935) Nevertheless the ventral nucleus must be taken into consideration here and it may be noted that the nucleus gives off a tract of fibres (fasciculus geniculatus descendens) which runs ventro-medially through the region of the subthalamus towards the hypothalamus

Two accessory optic tracts have been described in both normal and experimental material The anterior accessory tract leaves the main tract immediately behind the chiasma and is believed to terminate in the medial part of the subthalamus Some of its fibres have also been described as piercing the cerebral peduncle to reach the subthalamic nucleus of Luys The posterior accessory optic tract is better defined It leaves the main tract near the ventral margin of the lateral geniculate body and runs obliquely across the cerebral peduncle (where it is often known as the tractus peduncularis transversus) to the interpeduncular space It terminates in a mass of nerve cells close to the medial margin of the substantia nigra (nucleus opticus tegmenti) and probably in the subthalamic region immediately adjacent

According to some statements, the posterior accessory optic tract is only present in rodents and certain lower mammals It is quite distinct, however, in the ferret's brain

We have now to consider what elements of the lower visual centres have been involved in the experiments recorded in this paper

Superior colliculi and pretectal areas

The retinal connexions of the superior colliculi have been completely interrupted in experiments F 14, 15, 39 and 50, in the first two cases, the colliculi were completely isolated from the rest of the mid-brain In F 39 and 50, the retinal connexions of the pretectal area were also severed It appears, therefore, that these visual centres are not essential for the response which the animals showed to retinal stimulation

Lateral geniculate body (dorsal nucleus) and visual cortex

It is well established that in mammals the cells of the dorsal nucleus of the lateral geniculate body undergo atrophy after excision of the visual cortex On these grounds it has been suggested that they are entirely concerned with the projection of retinal impulses on to the cortex and have no other connexions The possibility remains however, that the cells of the geniculate body may be functionally connected to adjacent thalamic elements by short collaterals Therefore, while interruption of retinal fibres to the geniculate body inevitably cuts off all visual impulses to the cortex, destruction of the visual cortex does not necessarily prevent the geniculate body (at least before its atrophy is complete) from playing some part in mediating visual responses

In experiment F 23 the occipital lobes were removed Serial sections showed that the lateral geniculate bodies had undergone almost total cell degeneration Some normal cells, however, remained at the anterior extremity of the nucleus, and it is to be inferred, therefore, that a very small portion of the visual cortex had been left intact Although the animal from its general behaviour appeared to be quite blind as a result of this operation, it is necessary to recognize that a minute proportion of the geniculo-striate system had been left uninterrupted, for Lashley (1935) has shown that, in regard to brightness discrimination tests in rats, the effect of a lesion in the striate cortex has an all-or-nothing character, and that any part of the geniculo-striate system can be excited by impulses from the retina even though it is represented by only a few scattered normal cells in the lateral geniculate body

In experiments F 14 and 15, the occipital lobes of the cerebral hemispheres were also destroyed, but in both these cases a few apparently normal cells were found to be present at the extreme anterior end of the dorsal nucleus of the lateral geniculate body

In experiment F 22 the optic tracts were cut at the level of the ventral border of the lateral geniculate body On one side the lesion was not quite complete but serial sections indicate that the only fibres left intact are those passing behind the lateral geniculate body to the mid brain It thus appears that in this animal all visual impulses had been cut off from the dorsal nucleus of the lateral geniculate body and the visual cortex of both sides Except therefore for a few fibres passing to the mid brain on one side the only retinal fibres left intact are those which possibly terminate in the ventral nucleus of the lateral geniculate body and those which run in the accessory optic tracts

In experiment F 50 similar lesions in the optic tracts were complete with the one exception that a fragment of the ventral margin of the dorsal nucleus of the lateral geniculate body was left in relation to the optic tract on one side Inspection of serial sections shows that this fragment had undergone degeneration and was probably functionally insignificant but it is again necessary to note that the persistence of an occasional cell here does not entirely eliminate the possibility that a few isolated fibres of the geniculo striate system may have remained in action

These experiments thus show that the normal pituitary response to visual stimulation occurs in the absence of the superior colliculi or when all retinal impulses to any part of the mid brain have been interrupted They also show that it occurs when retinal impulses to the dorsal nucleus of the lateral geniculate body and the visual cortex have been completely interrupted while unless a few isolated normal cells in the dorsal nucleus of the lateral geniculate body provide the basis for adequate cortical stimulation they also suggest that a normal response can occur in the combined absence of the visual cortex and the superior colliculi Furthermore experiment F 50 strongly suggests that it can occur even after the interruption of retinal impulses passing to the dorsal nucleus of the lateral geniculate body visual cortex superior colliculi and pretectal area If this be so it follows that the normal response of the pituitary to retinal stimulation depends on impulses passing either to the ventral nucleus of the lateral geniculate body (which as noted above is considered to be a lateral extension of the subthalamus) or to the subthalamus by way of the accessory optic tracts

The further course by which such impulses might reach the pituitary gland are still uncertain A somewhat diffuse tract of fibres (fasciculus tuberis descendens) has been described by Nicolesco and Nicolesco (1929) running from the subthalamus into the tuber cinereum and Woollard (1932) has also demonstrated in Marchi preparations that the subthalamus

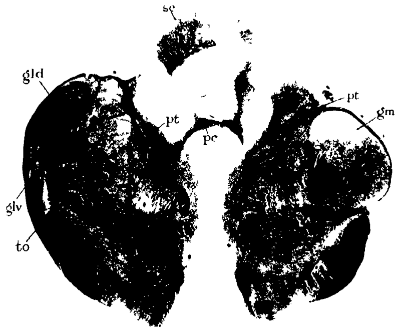

FIG 3 Transverse section through the thalamus and mid brain of ferret 15, showing the lesion involving the superior colliculi × 10 *gld* = lateral geniculate body (dorsal nucleus) *glv* = lateral geniculate body (ventral nucleus), *gm* = medial geniculate body, *pc* = posterior commissure *pt* = pretectal area, *sc* = superior colliculus, *to* = optic tract

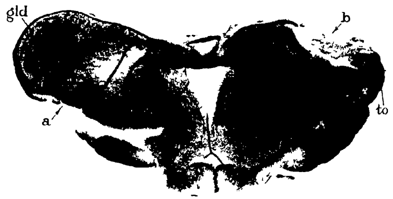

FIG 4 Transverse section through the thalamus of ferret 50, showing the lesions which have interrupted the left optic tract (*a*) and destroyed the dorsal nucleus of the right lateral geniculate body (*b*) *gld* = dorsal nucleus of lateral geniculate body (left), *to* = right optic tract × 9

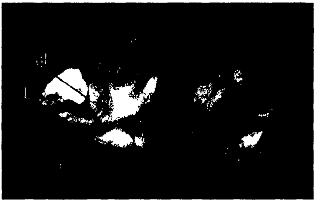

Fig 5 Lateral view of brain stem of ferret *22* showing the lesion in the left optic tract (*l*) Note that a small fasciculus of optic tract fibres passing to the mid brain has been left intact *gl* = lateral geniculate body, *sc* = superior colliculus, *to* = optic tract

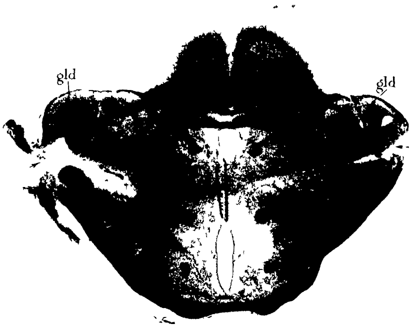

Fig 6 Transverse section through the thalamus and mid brain of ferret 22 × 8½ The position of the lesions in the optic tracts is indicated by arrows *gld* = dorsal nucleus of lateral geniculate body

is in fibre connexion with the hypothalamus Lastly fibre connexions have been described extending into the stalk of the pituitary gland from cell masses in the tuber cinereum These form part of the fasciculus whose main origin is the supra optic and paraventricular nuclei and which apparently terminates in the posterior lobe of the pituitary It thus appears that fibre paths are in existence through which retinal impulses can be relayed from the subthalamus to the posterior lobe of the pituitary gland

Whether the impulses passing by such a route can be relayed directly to the anterior lobe of the pituitary is highly doubtful The nervous connexions between the hypothalamus and pituitary (from the n supraopticus and n paraventricularis especially) are not generally assumed to extend from the pars posterior to the pars anterior although Pines (1925) states that they do In the rabbit according to Croll (1928) they do not extend beyond the pars intermedia Collin (1937) in reviewing the problem in general holds that some hypothalamic fibres do reach the secretory cells in the anterior lobe— mais semble t il en faible proportion In a recent paper Hair (1938) notes that a few fibres of the supraoptico hypophyseal tract reach the pars tuberalis and that occasionally fibres of similar origin can be traced to the anterior lobe In animals in which the pars anterior is completely separated from the pars posterior (e g cetaceans) there is of course no possibility of any direct passage of fibres down the infundibulum into the pars anterior In the circumstances there seem to be but slender anatomical grounds for supposing that the visual impulses concerned in gonadal activation are relayed to anterior lobe secretory cells along hypothalamic hypophyseal fibres It is just as likely that the retinal impulses affect the anterior lobe by way of vaso motor changes mediated by the hypothalamus or that in some way the impulses become transferred to the sympathetic nervous system by which the anterior lobe is richly supplied or that the neural impulses are transformed in the hypothalamus or posterior lobe to chemical stimuli which affect the secretory cells by way of the blood

A similar difficulty is encountered in explaining the occurrence of ovulation in rabbits after mating The initial stimulus is a nervous one It is transmitted to the anterior lobe of the pituitary where it is transformed into the chemical one which affects the ovaries Marshall and Verney (1936) found that the pituitary can be affected so that ovulation results by electrical stimulation through the brain and spinal cord Harris (1937) has shown that rabbits ovulate after direct stimulation of either the pituitary itself or the hypothalamus Haterius and Derbyshire (1938) also find that rabbits ovulate after direct stimulation of the hypothalamus Westman and Jacobsohn (1937), who have investigated these responses, noted that ovulation does not occur after stimulation of the brain if the stalk of the pituitary is first cauterized They also found that ovulation does not occur after copulation (which is the normal stimulus to ovulation in the rabbit), if the infundibulum is cauterized immediately after the mating These results suggest, as Westman and Jacobsohn point out, the direct transmission of nervous impulses along the hypothalamico-hypophyseal nervous connexions—in spite of the fact that in the rabbit these nerves have not as yet been traced into the pars anterior

In ducks the anterior lobe of the pituitary can be stimulated to produce its gonadotropic hormone by directing artificial light on to the gland, by way of a thin glass tube, after removal of the eyeballs (Benoit 1938) Whether or not this method of experimental stimulation has any connexion with the natural one is, however, doubtful Benoit has found that while ducks with hooded heads do not show a gonadal response to artificial illumination, ducks whose optic nerves have been divided or whose eyeballs have been removed do His conclusion, which is critically reviewed by Rowan (1938), is that the light acts through the ocular region and that the primary receptor is to be found in the region of the eyes The difficulty of defining the visual pathways by which the anterior lobe of the pituitary is stimulated would thus appear to be even greater in the duck than it is in the ferret

The question arises whether the neural mechanism involved in the activation of the gonadotropic function of the pituitary by retinal stimulation is identical with that which forms the basis of brightness discrimination It has now been well established that in lower mammals brightness discrimination is not dependent on the visual cortex This has been shown to be the case in the dog (Hilgard and Marquis 1935) and in the cat (Smith 1937) In the rat, Lashley (1935) suggests that there may be an equivalence of function for brightness vision between the striate cortex and the superior colliculi—either system may be destroyed without abolishing the capacity to react to brightness On the other hand, the experiments of Papez and Freeman (1930) showed that lesions localized to the superior colliculi and adjacent region of the brain-stem led to a retardation in relearning a light discrimination maze, and that the degree of retardation corresponded roughly with the severity of the lesion It may be noted that among his conclusions Lashley (1935) states that the accessory optic tracts and nuclei are not adequate for the formation of visual habits In general it would seem, therefore, that the visual mechanism for pituitary stimulation is not the same as that concerned in brightness discrimination

We have not attempted in our experiments to differentiate between the various cerebral lesions on the basis of the time-lag in the development of full oestrus With the exception of the two experiments in which both the visual cortex and the superior colliculi were removed, and in which the period of activation was slightly longer, full heat occurred in all the experiments after periods of illumination which do not differ materially from the normal The animals differed somewhat between themselves, however, in their ovarian responses to mating, although it was not possible to relate these differences to differences in their cerebral lesions Ovulation, as determined by rupture points and folding of the luteinizing granulosa, did not always occur, while luteinization of unruptured follicles was not infrequent Bissonnette and Bailey (1936) have also commented on the frequency of luteinization in unruptured follicles of ferrets which have been stimulated into oestrus by artificial illumination They attribute the phenomenon to the liberation by the anterior lobe of the pituitary of excess luteinizing hormone as a result of repeated coitus Hammond and Walton (1934) state that even normal ferrets in the breeding season may fail to ovulate after mating

SUMMARY

At the beginning of the non-breeding season lesions were made at various levels in the visual pathways of the brains of female ferrets The animals were then exposed, at the end of each day to bright artificial illumination for 6 hr 30 min in order to discover whether or not they would come into heat during the anoestrum Normal control animals, and ferrets whose optic nerves had been divided were exposed to the same conditions, while normal control animals were also kept under ordinary laboratory conditions

The ferrets whose optic nerves had been divided either did not come into heat at all, or came into heat much later than the control animals All the other animals came into oestrus either within or soon after the control period set by the normal animals exposed to additional light The experiments showed that the normal response to visual stimulation occurs in the absence of the superior colliculi, when all retinal impulses to any part of the mid-brain have been interrupted, and when retinal impulses to the dorsal nucleus of the lateral geniculate body and the visual cortex have been completely interrupted Unless a few isolated normal cells in the dorsal nucleus of the lateral geniculate body provide the basis for adequate cortical stimulation, it was also found that a normal response can occur in the combined absence of the visual cortex and the superior colliculi, while another experiment strongly suggested that it can occur even after the

interruption of retinal impulses passing to the dorsal nucleus of the lateral geniculate body, visual cortex, superior colliculi and pretectal area This being so, the conclusion is suggested that the visual response depends on impulses passing either to the ventral nucleus of the lateral geniculate body, or to the subthalamus by way of the accessory optic tracts

These results are discussed in relation to views on the exteroceptive stimulation of the pituitary in general and to the question of pituitary innervation in particular

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The mammary gland of the rhesus monkey under normal and experimental conditions

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[Plates 23–27]

The available information on the development of the mammary gland in monkeys and apes is limited, and can be classified as (*a*) that which concerns the growth of the gland in the normal animal, and (*b*) that which deals with its reactions to experimental endocrine stimulation

(*a*) NORMAL GROWTH OF THE MAMMARY GLAND

Turner and Allen (1933) report that mammary glands taken from mature virgin rhesus monkeys may occasionally possess an extensive lobule-alveolar system Some preparations from prepubertal females showed rich duct systems The mammary gland of the male rhesus was found by these workers to be restricted to a few ducts which rarely extend beyond the base of the nipple The mammary gland in the virgin rhesus has also been studied by Aberle (1934) who found that until a body weight of 2 5 kg is reached the mammary gland in the female is about the same size as in the male As the body weight rises from 2 5 to 5 kg, the gland rapidly increases in size, the most rapid growth occurring at puberty Aberle reports that although they were small in area, some preparations taken from prepubertal females were as densely glandular as glands taken from mature females She does not, however, state that these prepubertal glands showed true alveolar development It is of interest that while experimental investigation in many mammals has shown that progesterone in addition to oestrogenic hormone is usually necessary for the development of the mammary alveoli, it has been stated that the mammary alveoli in the monkey may grow under the influence of oestrogenic stimulation alone (see Nelson 1936 and also below) If oestrogenic stimulation has this effect in the monkey, Aberle's observations on prepubertal animals could be explained as being due to the abnormal production of oestrogen in the body before puberty

Zuckerman and Parkes (1932) studied the mammary glands of a series of female baboons (*Papio hamadryas* and *P porcarius*) The occurrence of cyclic changes, correlated with the menstrual cycle, could not be established They have been described as occurring in the mammary gland in other species during the oestrous cycle (e g mouse, Cogswell 1929, Cole 1933, heifer, Hammond 1927) This study of the baboon also provided no evidence that the alveolar tissue of the mammary gland in these animals develops cumulatively as a result of a succession of infertile cycles It appears to do so in the rhesus monkey (Turner and Allen 1933), in the rat (Turner and Schultze 1931), and probably in the virgin heifer (Hammond 1927)

Baboons continue lactating after the "lactation interval" (lactation anoestrus) which follows parturition (Zuckerman 1931, Zuckerman and Parkes 1932)

(*b*) EXPERIMENTAL DEVELOPMENT OF THE MAMMARY GLAND

The growth of the mammary gland of the monkey was first successfully demonstrated experimentally by Allen (1927) who observed that injections of oestrogenic hormone cause extensive duct development in ovariectomized female rhesus monkeys Later Turner and Allen (1933) reported that a slight degree of alveolar proliferation occurs if spayed female rhesus monkeys are given corpus luteum extract after a series of oestrone injections On the other hand alveolar growth is stated to have occurred in one normal male rhesus which was injected with relatively large doses of oestrogenic hormone alone—a finding which is also reported by Nelson (1936) Further observations on the effect of oestrin are recorded by Allen, Gardner and Diddle (1935) in an investigation in which female rhesus monkeys were used, and more recently by Gardner and van Wagenen (1938) who have reported on the responses of the mammary gland of both male and female rhesus monkeys to oestrogenic stimulation Alveolar development as a result of oestrogenic stimulation, without additional luteal stimulation, has also been reported in guinea-pigs (Haterius 1928) and in rats (Nelson 1935)

The aim of the present paper is to amplify the small amount of information that is available about the primate mammary gland The material studied was taken from both normal monkeys and monkeys which had been placed under various experimental regimes in connexion with other investigations

MATERIAL AND METHODS

The experimental male and female rhesus monkeys (*Macaca mulatta*) which were studied had been subjected to courses of injections with either "male" or "female" sex hormones or with various combinations of these hormones The preparations taken from "normal" mature females were in practically all cases obtained from cyclic females which had been subjected to operations in a study of the influence of neural lesions on the menstrual rhythm (Zuckerman 1938) Although the cycle during which the operation was performed was often somewhat shortened, the operations did not interrupt the regular succession of menstrual cycles in any of these animals

All the animals from which material for the present study was obtained were bought from dealers In no case, therefore, was the age known with certainty, nor was there any sure way of telling whether or not any female monkey was parous Estimates of age from body-weight-dentition relationships were made by use of data provided by Schultz (1933, 1935) Parity was judged by examination of the uterine vessels After the rigid exclusion of many specimens, we were able to obtain a representative series of preparations taken from virgin females in different stages of adolescence and maturity For comparison with this series there was one specimen which was known with certainty to have been parous

Macroscopic examination

The mammary glands were dissected at autopsy from the pectoral muscles, pinned out on a cork board and fixed for 24 hr by the application of pads of cotton wool soaked in alcoholic Bouin's fluid The preparations were then washed in 70% alcohol, overstained in Grenacher's borax-carmine, differentiated in 70% alcohol containing 5% HCl, taken up to absolute alcohol, and cleared and preserved in xylol No attempt was made to measure the shrinkage undergone during the process of preparation, but for practical purposes it may be assumed to have been approximately constant since all glands were treated alike According to Aberle (1934) the shrinkage which occurs during this method of preparation is small, and amounts on an average to 7%

Microscopic examination

Pieces of all mammary glands used in this investigation were sectioned, stained with haematoxylin and eosin, and studied histologically Sections were usually taken from both the peripheral and central zones of each gland

Measurement of mean mammary gland area

The mammary gland of the rhesus monkey is a flat or relatively flat sheet of tissue in all reproductive phases Measurements of the mean area of the paired glands accordingly provide one criterion of mammary development In view of the fact that Zuckerman (1932) observed no correlation between size and functional activity in glands taken from the green monkey (*Cercopithecus sabaeus*), caution is needed in interpreting data on mammary gland area, and it is necessary that such data should in all cases be supplemented by macroscopic and histological examination of the gland structure

The glandular area was measured by placing the flat stained preparation on the frosted glass lid of a box containing a powerful electric lamp A sheet of paper soaked in xylol was laid on the preparation and the purple-stained outline of the mammary gland was traced with pencil The areas were measured either with a planimeter or on squared paper The expression "mean mammary gland area" is used below to denote the mean area of the two glands of a pair

The mammary gland in the male monkey usually consists of a few short and uncomplicated ducts branching from the base of the nipple, and the mammary gland area in any given instance was taken as the area enclosed by a line joining the extremities of the ducts It is evident therefore that changes in mean mammary gland size in the male provide a less satisfactory criterion of gross changes in mammary development than they do in the female monkey

Preparation of figures

For photography, preparations were clipped between glass plates and immersed in a jar of xylol The magnification of each picture could be readily calculated by measurement of the image of a centimetre scale which was etched on one of the glass plates Silhouettes of portions of the glandular structures were traced on to thin Bristol board from suitably enlarged photographic prints

RESULTS

Growth of the mammary gland in comparison with general body growth

With the exception of the data presented by Aberle (1934), we are unaware of any study which deals with the growth rate of the mammary gland relative to that of the body It is therefore of interest to present an analysis of our own data from this point of view The relative rate of growth of the mammary gland in relation to general body growth was obtained by comparing mammary gland area (A) with a function which is proportional to the surface area (S) of the body The latter function was calculated from the body weight (W) by the use of the formula provided by Meeh (1879), $S = KW^{\frac{2}{3}}$ $W^{\frac{2}{3}}$ is accordingly a function which is proportional to the surface area

Twenty-six preparations taken from non-pregnant normal rhesus monkeys whose body weights ranged from 2200 to 7100 g were available for this study It was not known with certainty whether or not the adolescent and mature animals in this group had borne young prior to purchase

During a considerable part of the time during which the body weight of the rhesus monkey is increasing from 2000 to 6000 g , the mammary gland grows faster than the rest of the body (i e allometrically, Huxley and Teissier 1936) The simple allometric growth law (see Huxley 1932) is usually expressed as $y = bx^{\alpha}$, where $y =$ the quantity undergoing allometric growth, $x =$ the reference quantity, and b and α are constants the more biologically significant of which, termed the ‘ equilibrium constant”, is α Data relating to growth rates conform to this law if a straight line is obtained by plotting $\log y$ against $\log x$ The slope of such a straight line is equal to α Log_{10} mean mammary gland area is plotted against $\log_{10}$ (body weight)$^{\frac{2}{3}}$ in fig 1

Fig 1 shows that seventeen points (marked as black dots) lie very near to a straight line The best fit straight line for these points was obtained by the least square method and is shown in fig 1 The equation of this line is $\log_{10} A = 2\ 68 \log_{10}(W)^{\frac{2}{3}} - 4\ 86$ Of the remaining points, two shown in fig 1 by dots surrounded by circles lie very close to the line, while seven, shown in the diagram as black squares, deviate from it significantly

It follows from fig 1 that over the body-weight range 3400 to 6060 g the rate of increase in the area of the mammary gland agrees satisfactorily with the simple allometric law $A = 0\ 0000138\ (W^{\frac{2}{3}})^{2\ 68}$, or more simply, $A = 0\ 0000138\ W^{1\ 79}$, the equilibrium constant having the value 2 68 It is regrettable that the data for body weight values below 3000 and above

6000 g are so scanty, for the few points appearing in the left hand of fig 1 indicate that the equilibrium constant for low body weights would be considerably higher than 2 68

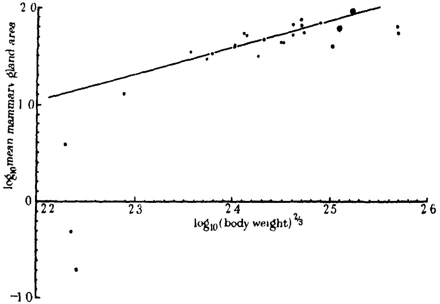

Fig 1 Relation between mean mammary gland area and (body weight)$^{\frac{2}{3}}$ in the female rhesus monkey Logarithmic plotting

Age changes in the structure of the mammary gland of the virgin rhesus monkey

Glands from seven virgin monkeys were available for this study The mammary glands of an eighth which had been autopsied some 2 months after she had weaned a baby were added to this series in order to compare the mammary gland during post-lactation involution with the virgin gland

Particulars relating to the eight rhesus monkeys comprising this series are given below The ovaries, which were sectioned serially, and the mammary glands were examined histologically

OM 252 Immature female Maximum body weight 1 4 kg The reproductive organs and sexual skin of this animal were completely inactive No permanent teeth had erupted and her age is estimated to have been approximately 18 months at death

The dimensions of the mounted and serially sectioned right and left ovaries are 7 5 × 5 0 × 3 2 and 7 0 × 4 0 × 3 4 mm respectively Neither ovary contains any follicle with dimensions greater than 1 5 mm , nor is there any trace of luteal tissue

The mean mammary gland area is 2 26 cm^2

OM 88 Immature female Maximum body weight 2 2 kg At autopsy the permanent incisors were in process of eruption The reproductive organs were immature and there was no evidence that this animal had ever menstruated Age at death is estimated as approximately 36 months

The dimensions of the mounted and serially sectioned right and left ovaries are 8 0 × 5 0 × 3 6 and 8 0 × 4 6 × 3 6 mm respectively Both ovaries contain numerous small atretic follicles and of the small healthy follicles that are present none has a diameter greater than 1 5 mm Neither ovary shows either recent or old luteal tissue

The mean mammary gland area is 3 84 cm^2

OM 251 Immature female Maximum body weight 3 08 kg This animal's central incisors were erupting at the time of her death and her age is estimated to have been about 38 months The animal was killed 39 days after the first pubertal changes had become apparent in her sexual skin and 13 days after the first signs of regression of the "blister" swelling of the scrotal lobes of the skin (Zuckerman, van Wagenen and Gardner 1938)

The dimensions of the fixed and mounted right and left ovaries are 9 0 × 5 0 × 3 5 and 7 5 × 4 5 × 3 6 mm respectively Neither ovary contains any luteal tissue, and each shows a wave of follicular growth Thus both contain several follicles with diameters varying between 1 and 3 mm , while the right ovary contains one follicle with a maximum diameter of 4 mm Some of these growing follicles are already showing signs of atresia

The uterus of this animal is in the phase of "interval" proliferation

The mean mammary gland area is 15 8 cm^2

OM 226 Immature female Maximum body weight 3 kg This animal's permanent incisors were erupting at the time of her death and her age is estimated to have been 38 months She was autopsied 27 days after the first day of her first menstruation and 53 days after the first pubertal signs of sexual skin activity had appeared

The dimensions of the mounted right and left ovaries are 7 1 × 5 0 × 3 4 and 7 5 × 6 5 × 4 6 mm respectively Both ovaries contain up to ten resting follicles with maximum diameters varying between 1 and 2 mm Neither ovary shows recent or old luteal tissue

The uterine glands are of the "interval" type, and the uterus is smaller and shows fewer signs of proliferation than does that of OM 251

The mean mammary gland area is 30 9 cm^2

OM 27 Adolescent female Maximum body weight 3 6 kg At the time of death the central incisors had erupted and the second molars were in process of eruption Microscopic examination makes it plain that the animal was nulliparous although it had undergone several menstrual cycles Her approximate age at death is estimated as 39 months

The dimensions of the sectioned right and left ovaries are 8 5 × 8 0 × 4 8 and 10 0 × 6 8 × 6 2 mm respectively Both ovaries contain numerous small resting follicles with maximum diameters varying between 0 5 and 1 5 mm The right ovary also contains the remains of a very degenerated corpus luteum, while the left shows three corpora of different ages, the most recent corresponding in form to the corpora aberrantia recently described by Corner (1936)

The mean mammary gland area is 29 5 cm^2

Mm 143 Adolescent nulliparous female Maximum body weight 4 0 kg Her permanent teeth were in a slightly more advanced stage of eruption than were those of OM 27 Her age was approximately 50 months

The dimensions of the right and left ovaries are 7 3 × 5 0 × 3 5 and 7 0 × 4 8 × 3 6 mm respectively Neither ovary contains any trace of luteal tissue The left ovary in addition to several 1 mm follicles contains one with a maximum diameter of 3 mm

The uterus is in an active phase of "interval" proliferation

The mean mammary gland area is 33 0 cm^2

Mm 116 Female not fully mature Maximum body weight 4 4 kg There was no evidence that this animal was parous Judging from her dentition she was slightly older at death than Mm 143

The dimensions of the right and left ovaries are 7 7 × 5 3 × 4 5 and 7 8 × 6 0 × 5 0 mm respectively Neither ovary contains any follicle larger than resting size The left ovary contains a corpus luteum in an advanced state of degeneration, and what appears to be an even more degenerated corpus is present in the opposite ovary

The uterine glands are of the interval type

The mean mammary gland area is 46 9 cm^2

Mm 56 Fully mature female Maximum body weight 5 7 kg This animal was unilaterally ovariectomized 6 months before autopsy Twelve months before her death she had given birth to an infant which she suckled for a period of approximately 10 months The lactation interval lasted only 3 months and she had regular menstrual cycles during the last 6 months of the nursing period The mean mammary gland area is 41 1 cm^2

The animals in this series have been listed in order of increasing estimated age Although the criteria of dentition and weight suggest that OM 27 was younger than Mm 143, the condition of her ovaries suggests that she was in a more advanced stage of sexual maturity In spite of this discrepancy it will however be seen that the increase in size of the mammary glands agrees fairly closely with the order of age in which the animals have been arranged

The series can be divided into two groups The first comprises those animals (OM 252, OM 88, OM 251, OM 226, Mm 143) whose ovaries show no signs of either existing or previous luteinization The second group is made up of those animals (OM 27, Mm 116, Mm 56) whose mammary glands at some time had been stimulated by luteal hormone

There is no essential difference in general structure between the prepubertal and pubertal glands in the first group of the series All these specimens show primary, secondary and tertiary ducts The only differences that are displayed are in mammary gland size and in the number and complexity of the lateral and end duct-buds Histological sections of selected pieces of these glands do not show alveolar formation (fig 14, Plate 23) The thickening at the periphery of the gland in, for example, OM 226, fig 5, consists of rapidly proliferating duct buds (fig 18, Plate 23) Turner and Gomez (1932) have commented on a similar appearance of the periphery in the mammary gland of the pubertal female albino mouse

It is of interest that the mammary glands of OM 252 are more complex in tubular structure than those of OM 88 in spite of the fact that its approximate age was at least a year less This fact indicates that considerable variation may occur in the time of onset of mammary growth and probably

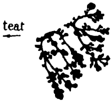

FIG 2 Silhouette of part of mammary gland of OM 252 × 2 6

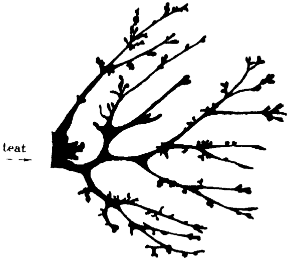

FIG 3 Silhouette of part of mammary gland of OM 88 × 2 9

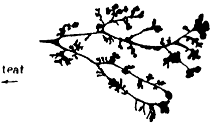

FIG 4 Silhouette of part of mammary gland of OM 251 × 2 3

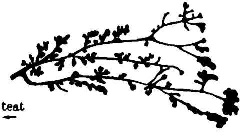

FIG 5 Silhouette of part of mammary gland of OM 226 × 1 8

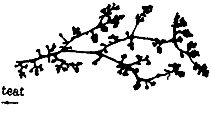

FIG 6 Silhouette of part of mammary gland of Mm 143 × 2 4

also in mammary growth-rate The specimens under discussion also make it obvious that the onset of development of the mammary duct system may precede the appearance of external signs of puberty This fact is also well demonstrated by a preparation of the mammary glands of a chimpanzee (M 96) which we have studied The chimpanzee in question weighed 15 4 kg

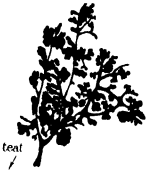

FIG 7 Silhouette of part of mammary gland of OM 27 × 3 1

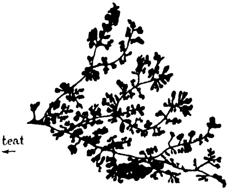

FIG 8 Silhouette of part of mammary gland of Mm 116 × 2 6

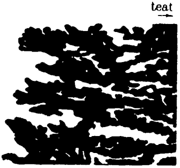

FIG 9 Silhouette of part of mammary gland of Mm 56 × 2 4

at the time of its death, and during its long stay in the London Zoological Gardens it had never shown any external signs of puberty (sexual-skin changes) Its age at death is estimated to have been four years Its internal reproductive organs were infantile and inactive, but its mammary glands already showed considerable duct development

The mammary glands of the three animals whose ovaries gave evidence of luteinization (OM 27, Mm 116, Mm 56) all show alveolar development This fact is more clearly demonstrated histologically than it is by examination of the "whole-mounts"—for glands which on macroscopic examination appear to be in the early stages of alveolar formation (e g OM 226, fig 5) may show no alveoli when studied microscopically

On macroscopic examination alveoli appear to be more abundant and the ducts more prominent in the mammary glands of OM 27 (fig 7) than in those of Mm 116 (fig 8) On the other hand microscopic examination shows that alveoli are much more abundant and more regularly disposed in lobules in Mm 116 (figs 16, 17, Plate 23) The ducts in Mm 116 are, however, in general much narrower than in OM 27, in which many appear to be distended with secretion, as do also a few alveoli

Our observations agree with those of Turner and Allen (1933), in showing that the mammary glands of presumed non-parous female rhesus monkeys may possess abundant alveoli

The lobules of alveoli in the mammary glands of Mm 56, the animal which is known to have been parous, are so well developed as to obscure the course of any of the ducts On microscopic examination the glands appear to be involuted (fig 15, Plate 23) but some of the alveoli, as well as several ducts, contain droplets of secretion In spite of their involution, however, the glands are much better developed than any others in the series Our observations thus suggest that while a succession of infertile cycles progressively develops the mammary gland in the rhesus monkey, especially after luteinization has occurred, maximal development in non-pregnant or non-lactating animals will be observed only in individuals which have at some time borne young

The male mammary gland

Male mammals possess rudimentary mammary glands and nipples, which in some species (e g rats, McEuen, Selye and Collip 1936, human beings, Andrews and Kampmeier 1927) may undergo pubertal development Under experimental conditions of endocrine stimulation the male gland behaves in the same way as does the female, and much of the experimental work on mammary development has therefore been carried out on male animals

Table I gives data relating to five normal control male rhesus monkeys

The mammary gland in the immature male monkey (OM 39) consists of a relatively uncomplicated duct system that radiates from the nipple (fig 10) Microscopic examination yields no evidence of active duct growth nor are any alveoli to be seen The mammary duct system in a somewhat older male (MM 11, fig 11), is less extensive but a few clumps of alveoli are to be seen on microscopic examination (fig 20, Plate 24) It does not seem, however, that either the ducts or the alveoli proliferate with age Thus

TABLE I THE MAMMARY GLAND IN THE NORMAL MALE RHESUS MONKEY

Monkey	Body weight at autopsy kg	Estimated age months	Mean mammary gland area cm ^{2}
OM 39	2 7	28	1 05
MM 11	3 9	39	Less than 1
OM 89	4 2	44	3 77
OM 90	5 0	50	Less than 1
OM 149	6 7	90+	Less than 1

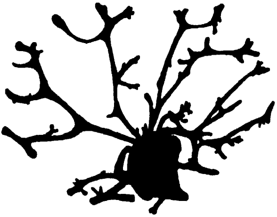

FIG 10 Silhouette of part of mammary gland of OM 39 × 4 8

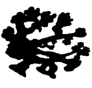

FIG 11 Silhouette of part of mammary gland of MM 11 × 2 1

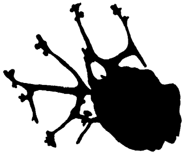

FIG 12 Silhouette of part of mammary gland of OM 90 × 4 1

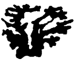

FIG 13 Silhouette of part of mammary gland of OM 149 × 1 2

in OM 90, fig 12, an adolescent male, and in OM 149, fig 13, a mature male, there is no further extension of the ducts, nor are any alveoli to be seen microscopically The mammary glands of OM 89 (fig 28, Plate 25) reveal, however, a condition of gynaecomastia The glands comprise an arborescent duct system associated with alveolar development (figs 21, 22, Plate 24) The glands are also larger than normal Gynaecomastia has also been observed in an adult male pig-tailed macaque, *M nemestrina*, in whom the mean mammary gland size was 20 cm ^{2} In this instance, however, there was no alveolar development, the glands consisting only of arborescent ducts

Gynaecomastia has often been observed in man (see Seifert 1920) and in the goat (Herre 1933, and Spann 1933) Its occurrence is of interest in view of recent demonstrations that androgenic substances can stimulate the development of the mammary apparatus (Selye, McEuen and Collip 1936, Nelson and Gallagher 1936, Astwood, Geschickter and Rausch 1937, Bottomley and Folley 1938) It is also of interest that the pubertal male rat normally possesses a mammary gland which is at least as well developed relatively as the glands are in the case of gynaecomastia noted above (Astwood and others 1937)

Effect of oestrone on the mammary gland of the male rhesus monkey

Table II gives data relating to twelve immature male rhesus monkeys and one immature Hanuman langur, *Presbytis entellus*, which were injected daily for periods varying between 6 and 484 days and with amounts of oestrone varying between 0 05 and 5 0 mg daily

TABLE II THE EFFECT OF OESTRONE ON THE MAMMARY GLAND OF THE MALE RHESUS MONKEY

Monkey	Maximum body weight kg	Estimated age months	Total amount of oestrone given mg	Period of treatment days	Oestrone per day mg	Mean mammary gland area cm ^{2}
OM 24	3 4	36	0 7	14	0 05	3 5
OM 43	2 8	30	0 7	14	0 05	Less than 1
OM 148	2 6	32	1 5	15	0 1	Less than 1
OM 153	2 4	19	2 2	22	0 1	Less than 1
OM 16	2 2	18	5 6	28	0 2	Less than 1
OM 151	2 5	22	3 7	37	0 1	Less than 1
OM 197	3 7	45	5 5	55	0 1	—
OM 101	2 9	30	6 2	62	0 1	3 4
MM 19	5 3	48	28 0	28	1 0	Less than 1
MM 1	4 6	43	30 0	6	5 0	Less than 1
MM 12	4 3	48	30 0	6	5 0	Less than 1
OM 115	2 8	28	36 8	368	0 1	26 3
OM 202 (*Presbytis entellus*)	5 0	—	290 4	484	0 6	14 6

Of these thirteen animals only OM 115, the monkey which was injected for 368 days, and OM 202, which was injected for 484 days, show definite increases in mammary gland area, it is likely, though not certain, that the glands of OM 24 and OM 107 had also increased in area

Only one (OM 197) of the animals shows any definite alveolar development The duct system in all except MM 12 appears to be actively proliferating (figs 26, 29, Plate 25) and several specimens show solid, and in some cases canalized, epithelial lateral and end buds (figs 24, 33, Plates 24, 26) No evidence of secretion was observed in any of the histological preparations In general, all the specimens give evidence of more active epithelial growth than was observed in any of the normal controls with the one exception of the instance of gynaecomastia (OM 89) Corroborative observations were made on three male green monkeys (*Cercopithecus aethiops sabaeus*) that were injected with oestrone for short periods

It appears that the extent of duct development varies directly with the duration of oestrogenic stimulation Thus the mean mammary area in OM 115, the animal which was injected daily for a year, is almost as great as is observed in mature females A similar result was obtained with a Hanuman langur (*Presbytis entellus*) which was given 600γ of oestrone daily for 484 days On the other hand large amounts of hormone given daily do not appear to affect the size of the gland if they are administered over too short a period (e g MM 1 and MM 12) Furthermore, the mammary glands of different animals appear to react differently to the same amount of oestrogenic stimulation (e g OM 24 and OM 43)

It may be noted that only one of five of our animals which received oestrone for 15 days or less failed to show active signs of mammary growth, whereas only one of nine male monkeys which were studied by Gardner and van Wagenen (1938) and which were given oestrogenic hormone for equally short periods, did show signs of proliferation

Our findings do not agree with those of Turner and Allen (1933), with those of Nelson (1936) and with those of Gardner and van Wagenen (1938), all of whom state that alveolar as well as duct development occurs in male monkeys which are injected with oestrone alone With the exception of Gardner and van Wagenen, these investigators do not give detailed observations, nor do they refer to histological examination of their material Macroscopic study of our own preparations suggests that true alveoli have developed (e g figs 26, 29, Plate 25), but this conclusion is not confirmed, except in one case (OM 197), by histological study Thus no alveoli are present in the mammary gland of either OM 202, the entellus monkey which was given oestrone daily for 484 days or in OM 115, the rhesus which was injected for a year Fig 32 (Plate 26) is a photomicrograph of a section of the mammary gland of OM 202 It demonstrates the proliferating duct system which, in places, is lined by a much thickened epithelium No alveoli are present Figs 23 and 25 (Plates 24, 25) are photomicrographs of the mammary gland of OM 197, which was injected with oestrone daily for 55 days Some scattered lobules of alveoli are present In view, however, of the similar appearance of one of the normal control specimens (OM 89, p 480) undue weight cannot be attached to this single case, especially in view of the absence of alveoli in the other twelve male rhesus monkeys which were given the same treatment

Gardner and van Wagenen (1938) state that in their experiments lobules appeared in the mammary gland of a male rhesus monkey after 22 weeks of treatment with oestrogenic hormone These lobules are described as being composed of well-formed alveoli, and it would seem that this specimen is similar to OM 197 discussed above

Effect of oestrone on the mammary gland of the female monkey

Our observations on the maturation of the mammary gland in the virgin female rhesus monkey indicate that in order to determine whether or not alveoli develop under any given set of conditions, it is essential to exclude animals that have experienced one or more phases of luteinization The following four monkeys were the only ones of a large number that had been treated with oestrogenic preparations which fulfilled this condition

OM 218 This immature female was prepubertal at the time of her death, when she weighed 2 3 kg She had not shown signs of sexual skin activity during her stay in the laboratory, and her age is estimated to have been 24 months

The dimensions of the mounted ovaries are 5 0 × 4 2 × 2 7 and 6 2 × 4 0 × 2 5 mm respectively Neither contains any trace of luteal tissue The maximum diameter of the largest follicle present is not as great as 1 mm

The mean mammary gland area was not determined

OM 152 Prepubertal female, maximum body weight 2 3 kg The age of this animal is estimated to have been approximately 21 months at the time of her death

The dimensions of the mounted and serially sectioned ovaries are 5 0 × 3 0 × 2 5 and 4 0 × 3 6 × 2 6 mm respectively Neither ovary contains follicles larger than 1 mm in diameter There are many atretic follicles but no sign of luteal tissue

The mean mammary gland area is 6 4 cm^2

OM 98 Adolescent female Maximum body weight 3 8 kg This animal had begun to experience menstrual cycles and her age was estimated as 44 months at death She was ovariectomized at a body weight of 3 6 kg and when her age is estimated to have been 32 months The operation took place on the first day of the second menstrual cycle experienced by her after her arrival in the laboratory

The mounted and serially sectioned ovaries measure 7 5 × 5 0 × 3 8 and 7 0 × 5 5 × 4 8 mm respectively Both ovaries show a wave of follicular growth and each contains several follicles, none of which has a maximum diameter greater than 2 mm There are many atretic follicles Neither ovary contains any trace of old or recent luteal tissue

The mean mammary gland area is 48 3 cm ²

OM 53 Adolescent female Maximum body weight 4 5 kg This animal had begun to experience menstrual cycles and her age is estimated to have been approximately 48 months at the time of her death Ovariectomy was performed on the 8th day of the third cycle which the animal had in the laboratory, and oestrone administration was begun 19 days after the operation At the time of the operation the animal's weight was 4 2 kg and her age is estimated to have been 46 months

The dimensions of the mounted and serially sectioned right and left ovaries are 6 5 × 5 0 × 4 4 and 5 5 × 4 5 × 4 2 mm respectively Neither ovary contains any follicle with a diameter greater than 2 mm There are many atretic follicles and some polyovular follicles but there is no trace of old or recent luteal tissue in either ovary

The mean mammary gland area is 26 9 cm ²

Table III gives a record of the total amount of oestrone given each of these animals and of the period of treatment Injections were given daily without interruption

TABLE III THE EFFECT OF OESTRONE ON THE MAMMARY GLAND OF THE FEMALE RHESUS MONKEY

Monkey	Maximum body weight kg	Total mount of oestrone given mg	Period of injections days	Mean mammary gland area cm ²
OM 53	4 5	3 09	40	26 9
OM 152	2 32	3 70	37	6 4
OM 218	2 30	4 80	48	Not measured
OM 98	3 80	36 50	365	48 3

Macroscopic examination of the "whole-mounts" of the mammary glands of this series of monkeys reveals a densely staining thickened peripheral region in each gland (fig 27, Plate 25) such as is seen in normal pubertal females Microscopic study shows that this thickened rim consists of rapidly proliferating ducts, of which many are dilated (fig 18, Plate 23) It is instructive to compare fig 27 (Plate 25) with the mammary gland of OM 88 (fig 3) which was an immature normal female that weighed approximately the same as OM 152 at death Signs of active mammary development are much more conspicuous in the gland of the oestrone-treated female

All the glands show a rich arborescent system of ducts, and with the exception of OM 152 and OM 218 (fig 19, Plate 23), they demonstrate fully formed alveoli OM 98, for example, consists of dense lobules of alveoli with little intervening stroma (fig 30, Plate 26) The alveoli are not dilated, but a few contain droplets of secretion (fig 31, Plate 26)

There is no evidence that any of the animals had experienced luteal stimulation before their ovaries were removed The presence of alveoli may therefore be ascribed to the action of the injected oestrone Consequently it seems that the female mammary gland is more responsive to oestrogenic stimulation than is the male gland

Effect of testosterone propionate on the male mammary gland

Macroscopic and microscopic preparations of the six male monkeys listed in Table IV were used in a study of the effects of male hormone on the male mammary gland The animals had been injected with testosterone propionate for varying periods Only OM 20, which was fully mature, had been castrated Details of the experimental treatment given each animal are arranged in Table IV, which also gives the size of the mammary glands at autopsy

TABLE IV THE EFFECT OF TESTOSTERONE PROPIONATE ON THE MAMMARY GLAND OF MALE RHESUS MONKEYS

Monkey	Maximum body weight kg	Estimated age months	Mean mammary gland area cm^2	Total amount of testosterone propionate g	Period of treatment days
OM 256	2 18	24	Less than 1	0 350	13
OM 239	1 71	16	Less than 1	0 200	14
OM 240	1 71	16	Less than 1	0 200	14
OM 250	2 58	24	Less than 1	0 400	14
OM 20	9 0	108 +	1 1	0 242	90
OM 190	4 98	48	1 1	1 69	151

In only two cases did the mammary gland at the end of treatment extend beyond the base of the nipple In contrast to the very slight macroscopic effects of the injections, histological study shows that pronounced changes were in fact produced by the androgenic stimulation These are best demonstrated in the two specimens (OM 190 and OM 20) which received the longest treatment

In OM 190 the main ducts are stunted but very dilated (fig 34, Plate 27) They are surrounded by apparently spherical lobules of alveoli, many of which are distended with globules of secretion The alveoli are as well developed as they are in any of our female specimens (fig 35, Plate 27) and much more so than they are in OM 89, the case of gynaecomastia discussed on p 481 There is very little interlobular stroma The duct epithelium is peculiar in so far as it is in many places heaped, and frequently shows papillomatous growth into the lumen These duct changes are even better demonstrated in OM 20, in whose mammary glands many mitoses can be seen They are also demonstrated in the specimens in which the period of male-hormone treatment was only 14 days (OM 239, fig 36, Plate 27) True alveoli were only demonstrated by OM 190

These observations are in keeping with those which were made on male rats by Astwood and others (1937)

It is of interest that the mammary glands of two mature female rhesus monkeys (OM 28, OM 77) whose ovarian activity was inhibited for periods of 114 and 210 days respectively (Zuckerman 1937) were not as involuted as would be expected in view of the involution of the uterus and vagina Numerous mitotic figures are to be seen in the glands, and many of the alveoli are distended with secretion

Effect of oestrone on the male mammary gland when given simultaneously with either testosterone propionate or progesterone

Histological preparations were made of the mammary glands of five immature male rhesus monkeys which were injected over a period of 14 days with both oestrone and testosterone propionate The actual details of the experimental treatment which these animals received has already been given in relation to the effects produced in the prostate (Zuckerman 1936) The ratio of the amount of testosterone propionate to oestrone administered varied between 36 1 and 7 1 Mammary glands of another seven immature monkeys which were given both oestrone and progesterone in a study on the prostate were also examined (Zuckerman and Parkes 1936, Table III) The ratio of progesterone to oestrone given in these experiments varied between 20 1 and 1 1 In none of these animals did the mammary tissue at autopsy extend beyond the base of the nipple In the first series of experiments oestrogenic effects prevailed when the ratio of testosterone propionate to oestrone was below 9 No changes which could be attributed to the testosterone propionate were observed in any of these specimens

Changes similar to those which are observed in animals injected with oestrone alone also prevailed in each of the second series of specimens No alveolar development was observed, and it may therefore be inferred that the normal effects of progesterone are not produced when the hormone is given simultaneously with oestrone in the ratios noted above As is well recognized, progesterone does cause such development after preliminary oestrogen treatment

The absence of carcinomatous changes after prolonged treatment with oestrone

Lacassagne (1932) observed the occurrence of carcinomatous changes in the mammary glands of male mice which were injected for prolonged periods with a solution of oestrone benzoate These findings, which have been confirmed by Burrows (1935) and others, are obviously of great importance in view of the increasing use of oestrogenic substances in clinical medicine Observations which we have been able to make on monkeys which received injections of oestrone for periods of one year and more are therefore not without interest Details of these animals are given in Table V In three cases, long periods of daily injections were separated by short periods in which no treatment was given

TABLE V PROLONGED PERIODS OF OESTRONE INJECTION IN MONKEYS

Monkey	Species	Sex	Days of experiment	No of days no oestrone given	Total amount of oestrone given mg
OM 98	*M mulatta*	Female	365	None	36 5
OM 95	,,	,,	398	103	23 01
OM 92	,,	,,	466	126	24 31
OM 74	,,	,,	938	391	44 7
OM 115	,,	Male	368	None	36 8
OM 202	*P entellus*	,,	484	None	287 8

Mammary cysts did not develop either during or at the end of the period of injections in any of these animals Furthermore, histological study failed to reveal any evidence for abnormal mammary growth (see figs 30, 31, 32, 37, 38, Plates 26, 27)

Mammary carcinoma does not develop in mice until after the end of periods of injection which, relative to the life span of the species, are much longer than those experienced by the monkeys listed in Table V Consequently, one cannot conclude that pathological mammary changes would not occur in monkeys if they were injected for longer periods than those indicated in the table Nevertheless, we feel that our results provide some reassurance regarding the possible dangers attending oestrogenic therapy, particularly as our observations have been made on a species which is more closely related to man than is the mouse

SUMMARY

1 Examination of the mean mammary gland area of twenty-six non-pregnant normal female rhesus monkeys shows that over the body-weight range 3400–6060 g the gland grows according to the simple allometric law

2 The first menstrual cycles of the rhesus monkey after puberty are usually anovular Until ovulation occurs, mammary glands grow by proliferation of their ducts, and alveoli do not appear to form until after luteinization has occurred

3 Normally the mammary gland of the male rhesus monkey consists of a relatively uncomplicated duct system which does not extend far beyond the base of the nipple One otherwise normal male showed a condition of gynaecomastia, with clumps of fully formed alveoli

4 Thirteen male monkeys were injected daily with varying quantities of oestrone for periods ranging from 6 to 484 days Only one of these animals (one that was injected for fewer than 15 days) failed to show duct proliferation Only one showed alveolar formation, no alveoli being present in the two animals which received the longest treatment (368 and 484 days respectively)

5 Only two of four female rhesus monkeys which were spayed before the beginning of the treatment with oestrone (37–365 days) showed alveolar formation

6 Testosterone propionate appears to stimulate stunted duct development and alveolar formation in male monkeys

7 No carcinomatous changes were observed in the mammary glands of any of six monkeys which were injected daily with oestrone either continuously, or for the major part of periods varying between 365 and 938 days

Our best thanks are due to Dr P White for his advice regarding the mathematical treatment of the data in the section on the growth of the mammary gland The monkeys used in this study were bought with the aid of a grant to S Zuckerman from the Medical Research Council The work was also supported by a grant to S Z from the Nuffield Medical Committee, Oxford The hormones that were used were generously provided by Dr Tausk of Organon, Oss, and Dr Karl Miescher of Messrs Ciba, Ltd

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DESCRIPTION OF PLATES

Plate 23

FIG 14 Photomicrograph of section of mammary gland of OM 143 No secreting alveoli are present, but the duct system shows signs of active proliferation × **24**

FIG 15 Photomicrograph of section of mammary gland of Mm 56 The mammary gland shows signs of involution after a period of lactation × 10

FIG 16 Lobules of alveoli of mammary gland in Mm 116, an animal which had experienced phases of luteinization × 24

FIG 17 High power view of alveoli in mammary gland of Mm 116 × 225

FIG 18 Section through thick peripheral rim of mammary gland of OM 253 showing actively proliferating ducts only × 24

FIG 19 Section of mammary gland of OM 218 showing ducts but no alveoli × 10

Plate 24

FIG 20 A duct and a clump of alveoli in the mammary gland of a normal male rhesus monkey, MM 11 × 81

FIG 21 Lobules of alveoli in the mammary gland of a normal male rhesus monkey, OM 89 The degree of alveolar formation in this specimen warrants the descriptive term "gynaecomastia" × 24

FIG 22 High power view of lobular development in OM 89 × 225

FIG 23 Clumps of secreting alveoli in the mammary gland of OM 197, a male rhesus monkey that had been injected with oestrone × 10

FIG 24 Lateral duct bud in mammary gland of OM 43, a male rhesus monkey that was being injected with oestrone × 150

Plate 25

FIG 25 A lobule of alveoli in the mammary gland of OM 197, a male rhesus monkey that was being injected with oestrone × 150

FIG 26 Photograph of "whole mount' preparation of mammary gland of OM 115, a male rhesus monkey that was injected with oestrone for a period of a year × 0 9

FIG 27 Photograph of "whole mount" preparation of mammary gland of OM 152, an immature female rhesus monkey which had been injected with oestrone for 37 days Note the densely staining peripheral zone × 1 9

FIG 28 Photograph of "whole mount" preparation of mammary gland of OM 89, a normal immature male rhesus monkey The excessive alveolar development in this specimen warrants the descriptive term "gynaecomastia" × 2 2

FIG 29 Photograph of ' whole mount" preparation of mammary gland of OM 24, an immature male rhesus monkey that was injected with oestrone for 24 days × 1 9

Plate 26

FIG 30 Section of mammary gland of an adolescent female rhesus monkey, OM 98, which was given daily injections of oestrone for a year × 24

FIG 31 High power view of a lobule of alveoli in the mammary gland of OM 98 × 225

FIG 32 Section of mammary gland of OM 202, an entellus langur which was injected with oestrone daily for 484 days No alveoli are present × 24

FIG 33 Section of mammary gland of OM 43, an immature male rhesus monkey which was injected with oestrone for 14 days The ducts are actively proliferating but no alveoli are present × 10

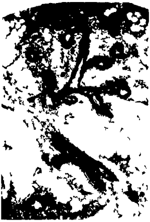
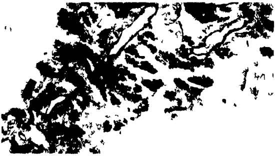


Fig 14



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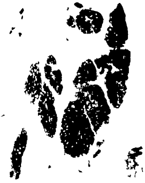

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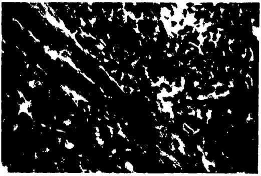

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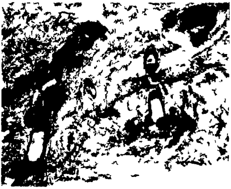

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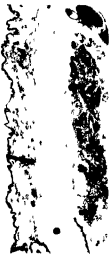

Fig 19

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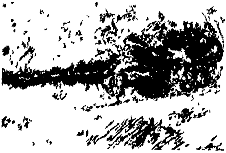
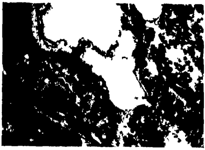


Fig 20



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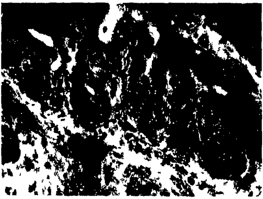

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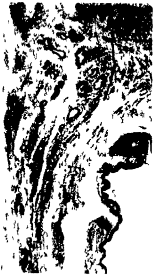

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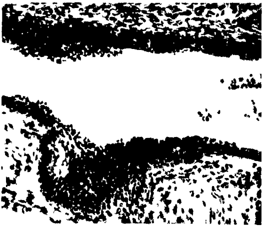
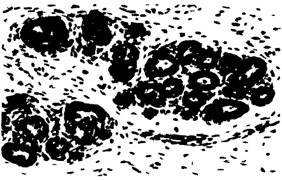


Fig 24



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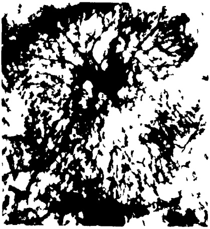

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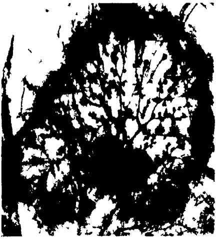

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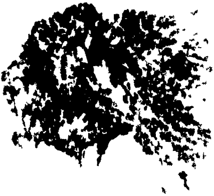

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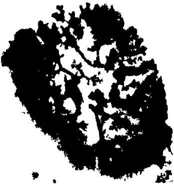

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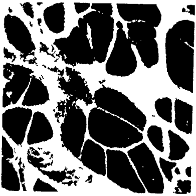

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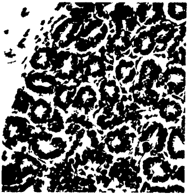

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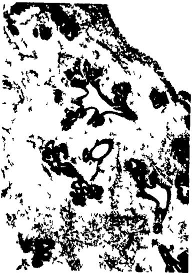

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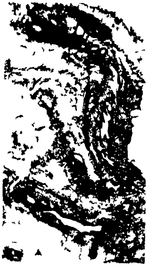

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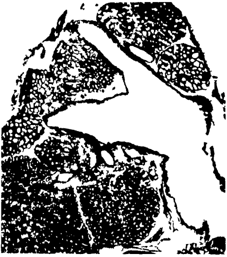
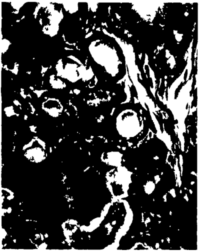


Fig. 34



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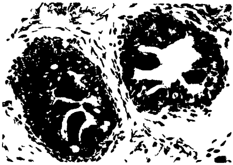

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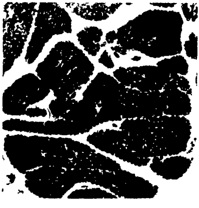

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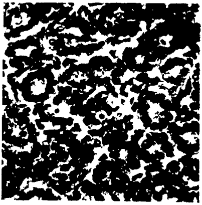

Fig. 38

Plate 27

Fig 34 Section of mammary gland of OM 190, an adolescent male rhesus monkey which was injected with testosterone propionate over a period of 151 days The ducts are distended and many lobules of alveoli are present × 24

Fig 35 High power view of alveoli in the mammary gland of OM 190 × 225

Fig 36 Transverse section of ducts in the mammary gland of OM 239, an immature male rhesus monkey which was injected with testosterone propionate over a period of 14 days × 150

Fig 37 Normal lobules of alveoli in mammary gland of a mature female rhesus monkey, OM 74, which was injected with oestrone a period of 938 days × 24

Fig 38 High power view of alveoli in mammary gland of OM 74 × 333

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Determination of lung volume a simple constant volume modification of Christie's method

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The total lung volume consists of the vital capacity plus the residual air Since the vital capacity may be measured directly, determination of the total lung volume depends on the measurement of the residual air Alternatively, the functional residual air may be measured and the figure added to the complemental air (terminology of Christie 1932) As the volume of reserve air expelled from the lungs in untrained subjects is liable to fortuitous variations, calculations of the residual air based on gas dilution at the end of a forced expiration are open to considerable error, for the same reason, determinations of the functional residual air at the end of an ordinary expiration may be inaccurate Van Slyke and Binger (1923) and Christie (1932) have shown that this source of error may be avoided by taking graphic spirometer records of respiration during the period of mixing Since the dilution of the lung gases is achieved by ordinary quiet respiration, this method requires no special respiratory efforts and is thus eminently suitable for clinical investigation

Determination of lung volume by Christie's method, the underlying principle of which is exceedingly simple, involves only the recording of an ordinary spirometer tracing and the analysis of the oxygen and nitrogen content of the spirometer gas at the end of a period of rebreathing The essence of the method is as follows

The dead space of the spirometer with its attached tubes is estimated with the spirometer bell in the zero (empty) position A measured amount of oxygen, say 4 l, is added to the spirometer The subject is switched into the spirometer circuit and a record is made in the usual way At the end of 7 min the subject is disconnected, and it is assumed that in this time complete equilibrium has been reached between the gases in the patient's lungs and in the spirometer After allowing diffusion of the gases to take place in the spirometer, a sample is taken and analysed It is assumed that the volume of nitrogen in the closed lung spirometer system remains constant, and the calculation is made as follows

Let x be the volume of air in the lungs at the time of switching the patient into the circuit As a convenient example let us suppose the spirometer dead space to be 2 l, the final percentage of nitrogen in the spirometer (as found by analysis) to be 50, and the final spirometer volume at the same phase of respiration as that at the beginning of the record to be 3 l from the zero mark The latter figure added to the spirometer dead space volume gives a total final spirometer volume of 5 l For a reason to be stated presently, the nitrogen content of the air in the lungs may be assumed to be the same as that of the inspired air, i e 79 1 % N_2 at the beginning of the experiment and 50 % at the end Now, the volume of nitrogen in the system at the beginning of the rebreathing period is $0\ 791 \times 2 + 0\ 791x$ l, and this, by assumption, is equal to the volume of nitrogen in the system at the end, i e $0\ 50(x+5)$ l

Thus
$$1\ 582 + 0\ 791x = 0\ 50x + 2\ 5, \tag{1}$$
$$0\ 291x = 0\ 918,$$
$$x = 3\ 16\ \mathrm{l}$$

Although it is well known that the percentage of nitrogen in the lungs is more than 79 1 (except in the event of the respiratory quotient being unity), Christie made the reasonable assumption that the relative increase in the percentage of nitrogen taking place in the lungs would be approximately the same whether the inspired air contained 79 1 or 50 %, provided that the respiratory quotient remained unaltered The errors introduced by the assumption of an unaltered concentration of nitrogen in the lung air as compared with the inspired air appear on both sides of equation (1) and are almost exactly cancelled out. In this way the necessity for determination of the nitrogen percentage in the lung air is avoided.

A recent and careful study by Lassen, Cournand and Richards (1937) has, however, shown that Christie was not justified in making this assumption, and the value of his otherwise extremely useful and easy method is accordingly in doubt. By alveolar air sampling, they showed that in a closed lung-spirometer system with a steadily *diminishing* volume there was no nitrogen concentration in the lungs, as the lungs constitute a virtual reservoir of air, the nitrogen content of which approximates to that of air breathed perhaps half a minute previously. A tidal inspiration of 500 c.c. is obviously inadequate completely to change the composition of, say, 3000 c.c. of functional residual air. In the closed system with diminishing volume, the alveolar air at the end of a few minutes' rebreathing has been shown to have a lower nitrogen percentage than the air in the spirometer at the same moment. As alveolar air may contain as much as 81% of nitrogen when the patient is breathing atmospheric air, the corrected equation (1) might be

$$\begin{aligned} 1.582 + 0.81x &= 0.49x + 2.5, \\ 0.32x &= 0.918, \\ x &= 2.87 \text{ l.} \end{aligned}$$

Christie's original method thus tends to give results which are too high. With large volumes of functional residual air the lung storage effect becomes more and more marked and the error of the method is exaggerated. By the somewhat laborious method of analysing the alveolar air just before and at the end of the period of rebreathing, Lassen, Cournand and Richards (1937) were able to eliminate this error. The deviation of Christie estimations from the true value is shown by the circles in fig. 1. It is seen that the discrepancy is most pronounced when the functional residual air exceeds 3 l.

The procedure adopted by these authors trebles the number of gas analyses required, and the method becomes entirely unsuitable for clinical investigations, in which alveolar air sampling is notoriously unreliable. As they have shown, the difficulties of applying the assumptions made by Christie would not arise but for the fact that the volume of gas in the spirometer is steadily diminishing, thereby producing a continually rising percentage of nitrogen in the inspired air, and making a final equilibrium impossible of achievement. It is therefore evident that the addition of oxygen at a uniform rate to the spirometer so as to balance its removal by the lungs, thus keeping the nitrogen percentage of the inspired air constant, would obviate this difficulty The lung air would come into equilibrium with a new atmosphere of constant composition, lung "nitrogen lag" would be abolished, and the original Christie equation could be used without appreciable source of error

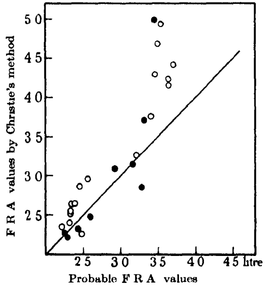

FIG 1 The probable functional residual air values estimated by Cournand and others by alveolar sampling (circles) and by the constant volume method (dots) are plotted against the results in the same subjects obtained by Christie's method Agreement of the results is shown where the points cluster along the diagonal Christie s method gives results markedly above the true figures with values over 3 1

A number of determinations of lung volume were made on a series of subjects under identical conditions by Christie's original method, and by the 'constant volume modification The results obtained are shown in Table I and plotted (dots) in fig 1 It is seen that, while there is reasonable

TABLE I DETERMINATIONS OF FUNCTIONAL RESIDUAL AIR BY CHRISTIE'S METHOD AND THE CONSTANT VOLUME METHOD

Constant vol	Christie	Constant vol	Christie
2 41	2 33	3 30	3 70
2 91	3 10	2 58	2 48
3 15	3 15	2 28	2 21
3 46	5 00	2 23	2 28
3 26	2 85	2 36	2 13

agreement between the two methods when the functional residual air is less than 3 l, Christie's method gives estimations which are too high at values above this figure—a finding very much in accordance with that seen on comparing the original method with the modification devised by Cournand and others Clearly, then, the error introduced by Christie's assumption can be eliminated by adopting either the procedure of Lassen, Cournand and Richards or the method described in this paper, but the great advantage of the latter lies in the fact that it is a simple modification of technique, which introduces no difficulties of additional gas analyses or gas sampling

APPARATUS

The apparatus we have used is the Knipping type of spirometer with a circulating air pump as supplied by C F Palmer, Ltd The pump in this machine circulates the air at rates up to 40 l/min In this way rapid and efficient mixture of gases is assured, and there is no possibility of incomplete mixing such as might result from the addition of oxygen to the

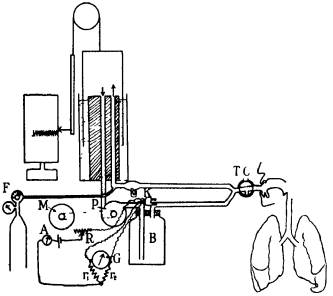

FIG 2 Modified Knipping spirometer for determination of lung volume The patient is connected to the apparatus by the three way cock (*T C*) and the air in the spirometer is kept circulating by the pump *P* driven by the motor *M* Bottle *B* is filled with cal soda chips to absorb CO_2 The volume of the whole system is kept constant by running in oxygen at a uniform rate from a cylinder through a calibrated needle valve *F* A katharometer chamber *K* is inserted in the circuit, connected through a battery, variable resistance *R* and ammeter *A* to a galvanometer *G*

more common type of apparatus, in which the air circulation is maintained by the patient s own respiratory efforts Also, at the end of the experiment there is no delay due to waiting for the gases to diffuse and mix through the tubes

The apparatus supplied has to be modified The usual wash-bottle for the absorption of CO_2 is removed and replaced by a bottle of cal-soda chips This removes CO_2 with much greater efficiency The amount of water in the spirometer is reduced very considerably by the insertion of a solid paraffin core, as suggested by Christie while the water level may be kept constant, after estimation of the dead space, by the use of a small syphon manometer

We have also found the insertion of a katharometer chamber in the tube leading the inspired air from the CO_2 absorbing bottle to be of considerable value The katharometer is connected to a sensitive galvanometer, the needle of which settles down to a constant level when mixing is complete throughout the lung-spirometer system The galvanometer may be calibrated to give an approximate reading of the percentage of oxygen in the spirometer, but owing to the sensitivity required, zero fluctuations render it impossible to use this method as a substitute for analysis

TIME REQUIRED TO REACH EQUILIBRIUM

Fig 3 shows examples of the changes in the galvanometer reading over the last few minutes of the rebreathing period in a few subjects It is seen that the needle may not settle down to its final value until 6 min have

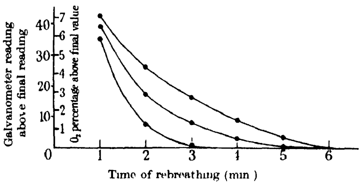

FIG 3 Three examples of the rate of change of oxygen percentage in the inspired air as gauged from the katharometer The galvanometer needle lags about half a minute after the actual change in gas composition has taken place so that complete equilibrium is actually reached sooner than indicated

elapsed Equilibrium is invariably reached within this time in normal subjects, and in a few instances, e g where no change in the galvanometer reading takes place after the fourth minute, it is achieved sooner As the katharometer current was adjusted to give a needle deflexion of 6 divisions for a change in oxygen concentration of 1 %, the rate of change of the oxygen percentage in the inspired air may also be gauged from the curves

In the absence of a katharometer it is wise, therefore, to continue the record into the seventh minute This is slightly longer than the time suggested by Van Slyke and Binger (1923), viz that 5 min was adequate for the achievement of equilibrium

TECHNIQUE OF DETERMINATION

In routine work the patient is connected to the spirometer and an ordinary tracing of respiration taken During this procedure the vital capacity, complemental air and reserve air are recorded separately In order to avoid errors introduced by lack of effort on the part of the subject, at least two determinations of vital capacity are made From this record the rate of oxygen consumption is also estimated

The apparatus is now washed out with atmospheric air led in through a pipe from the outside This may conveniently be carried out by the circulating air pump, and 5 min washing is usually adequate The spirometer bell is then put in the zero position and 4 l of cylinder oxygen of known composition are added By means of a calibrated fine-flow adjustment, sufficient oxygen is added at a uniform rate to balance its rate of consumption by the subject The subject breathes normally in the closed circuit for about 7 min until he has come into complete equilibrium with the new atmosphere An unchanging composition of the air in the circuit is indicated by a steady reading on the galvanometer connected to the katharometer in the circuit The patient is then disconnected and the pump allowed to run for a minute or so to remove any accumulated CO_2 in the apparatus The final CO_2 percentage, which may be estimated by the diminution in spirometer volume subsequent to switching the patient out of the circuit, should not be allowed to rise above 0 5 % Samples are then taken and analysed for their nitrogen and oxygen content A Van Slyke manometric apparatus may suitably be used to make these analyses

The spirometer volume at the end of the experiment is found by ruling the usual line through the expiratory positions along the base of the respiratory record ("resting respiratory level") The exact position of this line can usually be determined easily, but occasionally the level may be irregular and an absolute line may be difficult to establish In such cases, Christie adopted the criterion that the record could be accepted only if the possible error did not exceed 50 c c If it is impossible to determine accurately the general slope of the record to within 100 c c , the record should certainly be discarded for the purposes of this calculation—an error of this magnitude altering the final result appreciably Thus, substituting 4 9 for 5 0, in equation (1) we have

$$0\ 791x + 1\ 582 = 0\ 50(x + 4\ 9),$$

$$0\ 791x + 1\ 582 = 0\ 50x + 2\ 45,$$

$$0\ 291x = 0\ 868,$$

$$x = 2\ 98$$

In this example, therefore, a discrepancy of 100 c c in the estimated position of the "resting respiratory level" makes a difference of 0 18 l in the result

CORRECTIONS

The subdivisions of lung volume other than the functional residual air are measured directly from the spirometer tracings of complementary air, reserve air and vital capacity No corrections for temperature need be applied as these measurements represent closely the true volume changes in the lungs This fact can be demonstrated readily by taking a full inspiration from the spirometer and then holding the chest in the position of maximum possible inspiration, as the air in the lungs is warmed its expansion is recorded on the spirometer tracing By this means it is found that the time required for full warming of the air is about half a minute The change of temperature occurring during the few seconds normally occupied by the recording of vital capacity and its subdivisions may be regarded as negligible

Several corrections however must be made to the functional residual air value as calculated For convenience we shall discuss these corrections in relation to a functional residual air value of 3 l

(1) *Temperature correction* The calculation of x (equation (1)) is made as though the air in the lungs is at the temperature of the air in the spirometer at the end of the experiment This temperature is usually about 20° C, and at 37° C the volume of air in the lungs (functional residual air) would obviously be $3\ 00 \times 310/293$ or 3 17 l The air in the lungs is thus about 170 c c greater than that actually calculated

(2) *Vapour pressure correction* The calculation as made is only correct in so far as an even distribution of water vapour may be assumed throughout the system Such an assumption is of course not justified, the saturated vapour pressure of water in the spirometer at 20° C being 17 mm Hg and in the lungs at 37° being 47 mm Hg, a difference of 30 mm Hg With a functional residual air of 3 l the true lung volume corrected for complete saturation with water vapour would be 760/730 × 3 or 3 12 l

(3) *Dead space correction* The calculated volume includes that between the tap of the spirometer mouthpiece and the alveoli, that is the whole respiratory dead space as well as the additional dead space provided by the spirometer mouthpiece The mouthpiece dead space in the apparatus we used was 60 c c On the average about 150 c c should be subtracted for the true respiratory dead space, but this is only an average figure, and for most purposes it is convenient that the lung volume should include this dead space

(4) *Error introduced by the escape of nitrogen from the blood during the mixing of lung and spirometer air* During the course of the experiment the nitrogen content of the gases in the lung is steadily diminishing and consequently an appreciable quantity of the nitrogen dissolved in the blood passes into the alveoli, the volume of nitrogen added in this way to the lung spirometer system has been calculated by Christie, from the data of Hill, Long and Lupton (1924) and of Campbell and Hill (1931), to be about 65 c c To keep this figure approximately constant and thus obtain comparable results the estimates should always be made at the same spirometer volume, and the standard duration of the rebreathing period should be seven minutes The error created by the addition of 65 c c nitrogen during the rebreathing period is shown by adding this figure to equation (1)

$$1\ 582 + 0\ 791x + 0\ 065 = 0\ 50x + 2\ 5,$$

whence $x = 2\ 94$ l or 0 22 l less than the calculated value

When the calculated functional residual air is approximately 3 l and the temperature of the spirometer gases is 20° C the value of these errors may be tabulated as follows

	Add c c	Subtract c c
Temperature correction	170	
Vapour pressure correction	120	
Escape of nitrogen from the blood		220
Dead space of mouthpiece		60
Dead space of respiratory passages (average)		150

TABLE II DUPLICATE DETERMINATIONS OF FUNCTIONAL RESIDUAL AIR (ARRANGED IN ORDER OF MAGNITUDE OF THE DIFFERENCES)

No	First determination	Second determination	Difference (D)	D^2
1	3 08	3 08	0 0	0 0
2	3 10	3 12	0 02	0 0004
3	2 95	2 97	0 02	0 0004
4	2 35	2 32	0 03	0 0009
5	3 45	3 50	0 05	0 0025
6	2 90	2 95	0 05	0 0025
7	4 55	4 50	0 05	0 0025
8	3 21	3 28	0 07	0 0049
9	2 60	2 68	0 08	0 0064
10	4 00	3 91	0 09	0 0081
11	3 20	3 10	0 10	0 0100
12	2 42	2 30	0 12	0 0144
13	2 45	2 58	0 13	0 0169
14	2 91	3 04	0 13	0 0169
15	3 09	3 24	0 15	0 0225
16	2 07	1 90	0 17	0 0289
17	3 70	3 87	0 17	0 0289
18	2 47	2 66	0 19	0 0361
19	3 40	3 60	0 20	0 0400
20	1 50	1 70	0 20	0 0400
21	3 30	3 50	0 20	0 0400
22	4 27	4 07	0 20	0 0400
23	3 00	3 22	0 22	0 0484
24	2 62	2 38	0 24	0 0576
25	2 44	2 20	0 24	0 0576
26	2 45	2 20	0 25	0 0625
27	3 20	3 47	0 27	0 0729
28	3 82	4 09	0 27	0 0729
29	3 03	3 31	0 28	0 0784
30	2 10	1 80	0 30	0 0900
31	3 30	3 00	0 30	0 0900
32	3 26	3 58	0 32	0 1024
33	3 38	3 04	0 34	0 1156
34	3 15	2 80	0 35	0 1225
35	3 24	3 70	0 36	0 1296
36	2 62	2 24	0 38	0 1444
37	3 30	2 90	0 40	0 1600
38	2 37	1 95	0 42	0 1764
39	3 42	3 85	0 43	0 1849
40	1 75	1 30	0 45	0 2025
41	2 50	2 97	0 47	0 2209
42	2 20	2 71	0 51	0 2601

Sum of the absolute differences	9 22
Mean difference (9 22/42)	0 219 1
Mean difference from mean value of each pair	0 110
Sum of squares of the absolute differences	2 8127
Variance of absolute differences (2 8127/42)	0 0670
Whence S D	0 259
Standard deviation of the differences from the mean, or standard error of a single observation	0 259/$\sqrt{2}$ or 0 183 1

Thus if we include in the lung volume the dead space of the respiratory passages it is seen that the total correction required is +0 01 l Similar calculations show that the total correction at functional residual air values of 2 and 4 l are minus 0 04 and plus 0 02 l respectively

ACCURACY OF THE METHOD

In Christie's publication a series of determinations on three normal trained subjects showed a high degree of accuracy There is no doubt that equally good results could be obtained under these conditions by the present method The real value of a method depends, however, on its ease of application, and its accuracy can be more usefully assessed from a study of the results obtained on a series of unselected individuals who had no special training as subjects of respiratory experiments The duplicate determinations shown in Table II were made in a series of 42 male members of a mining population who had never previously seen respiratory apparatus

The accuracy of the method compares well with the results obtained by Christie in his three normal subjects in which the average deviation from the mean was 0 093 l as compared with our value of 0 110 l

This work was carried out with the aid of an expenses grant from the Lawrence Fund of the Royal Society

SUMMARY

1 Christie's simple method of lung volume determination by the use of a spirometer and involving only a single gas analysis is liable to considerable error, especially with functional residual air values above 3 to 3 5 l

2 Modifications in technique are suggested which apparently eliminate the error while retaining its simplicity and ease of application

3 A series of duplicate determinations on forty two untrained subjects reveals the standard error of a single determination to be 0 183 l

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The lung volume and its subdivisions in normal males

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P D'Arcy Hart, *Member of Scientific Staff, Medical Research Council*
and J McMichael, *Johnston and Lawrence Research Fellow, Royal Society*

A method of determining the lung volume has been described in the preceding paper (Herrald and McMichael 1938) During the course of an investigation on chronic pulmonary disease in the South Wales coalfield,* sixty-six normal adult male subjects were studied as controls, and the results of this part of the investigation are here presented The only previous work on comparable numbers was carried out in Rochester, New York, by Hurtado and his co-workers, who examined a hundred young adult normals (fifty males and fifty females) and, more recently, fifty middle-aged normal males, in the recumbent position (Hurtado and Boller 1933, Hurtado, Fray, Kaltreider and Brooks 1934, Kaltreider, Fray and Hyde 1938) They also contrasted the results on males with ten subsidiary observations, made in the sitting posture, in each of the two series (Hurtado and Fray 1933*b*, Kaltreider, Fray and Hyde 1938) These workers point out that no satisfactory standards of normality for total lung volume together with its subdivisions had previously been available Their own standards would be generally acceptable were it not for the fact that doubt has been thrown on the validity of the method used (Lassen, Cournand and Richards 1937) The source of error in the method is briefly discussed in the preceding paper, and it is shown to be most pronounced at functional residual air values of over 3 5 l Since most normal values of this measurement are below this figure it is not surprising that our present results show remarkable general agreement with the figures of the Rochester workers

1 Terminology

Throughout this paper we use the following terminology

Residual air is the amount of air remaining in the lungs after the fullest possible expiration

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* Investigation in progress by the Industrial Pulmonary Diseases Committee of the Medical Research Council

Functional residual air is the amount of air remaining in the lungs after normal expiration. In the spirometer record of respiration the position reached after a normal expiration is called the ***resting respiratory level***.

Complementary air is the volume of air inspired from the resting respiratory level to the position of maximum inflation. It includes the tidal air.

Reserve air is the amount of air expired from the resting respiratory level to the position of maximum deflation. Reserve air plus residual air equals the functional residual air.

Vital capacity is the amount of air expired in the fullest possible expiration following the deepest possible inspiration. Vital capacity usually equals the sum of the complementary and reserve airs.

Total lung volume is the sum of the residual air and the vital capacity.

2 METHODS

(a) *Lung volume determinations*

The technique adopted was that described in the preceding paper. By this method the possible error of the original Christie technique is avoided. The determinations were made with the subject under resting conditions sitting on a high-backed chair with the head supported on a cushion.

The sitting, rather than the recumbent, posture was decided upon as being more suitable for standard use, for the following reasons:

(1) The sitting posture is a nearer approach to the standing position commonly used in this country for chest radiography.

(2) Most other types of physical examination and chest measurement are preferably made with the subject sitting up.

(3) The respiratory effort involved in recording vital capacity is made with greater ease and freedom in this position.

The value of the *functional residual air* as given represents the true volume of the air in the lungs, taking into account the effect of temperature and water vapour. The *vital capacity*, *reserve* and *complementary airs* were obtained directly from the spirometer tracings, the vital capacity being recorded at least twice. No corrections for temperature or vapour pressure need be applied to these spirometric readings (see Herrald and McMichael 1938). The *residual air* was calculated by subtracting the reserve air from the functional residual air. The *total lung volume* was obtained by adding either the sum of the reserve and complementary airs or the vital capacity—whichever value was the greater—to the residual air.

(b) *Physical and radiological measurements*

The ***standing*** and ***stem heights*** and the ***weight*** were taken with the subjects stripped to the waist and without boots, the height to the nearest $\frac{1}{4}$ in. and the weight to the nearest pound. For the stem height, the platform of the height standard was placed on a chair and the subject instructed to sit on it with the buttocks and the back of the head pressed against the upright scale, and with the top of the head horizontal this method ensured a uniform sitting position

The *surface area* was obtained from the standing height and weight by use of Boothby and Sandiford's (1921) nomograph, and from the surface area and Du Bois's standards for normal basal calories per square metre per hour for males of the particular age the *normal total basal calories per day* were obtained by means of the same nomograph

The *chest circumference* was measured on full inspiration, horizontally ½ in below the nipple level, with the arms at the sides Both this and the *circumferential expansion* are given to the nearest ¼ in

The *area of the lung fields* and the *radiological chest volume*, terms introduced by Hurtado and Fray (1933*a*), were obtained as follows The area of the lung fields (area of heart included) was determined by means of a planimeter from an X ray film taken at a focal film distance of 54 in on full inspiration and in the standing position The radiological chest volume was calculated by multiplying the area of the lung fields by the external antero posterior chest diameter The latter measurement, to the nearest ¼ in, was taken on full inspiration by a pelvimeter placed horizontally ½ in below the nipple level in the mid line

The *radiological chest height/width* × 100, an index also introduced by Hurtado and Fray, was obtained from the same X ray film The height was determined by measuring the vertical distance from the dome of the diaphragm to the plane of the neck of the first rib on both sides and taking the mean The width was obtained at the level of the ninth rib posteriorly, the measurement being taken from the inner aspects of the ribs

3 CRITERIA OF NORMALITY AND PHYSICAL MEASUREMENTS OF SUBJECTS EXAMINED

The sixty-six subjects of this study were selected from the normal members of the working population of an anthracite colliery in Carmarthenshire, the great majority being of Welsh parentage Their ages ranged from 19 to 63 years, and their heights from 60 to 71 in Both surface and underground workers were included

The subjects conformed to the following criteria

(1) X rays showing normal lung fields and no definite enlargement of the heart shadow, judged by the cardio thoracic ratio

(2) Exercise tolerance classed as normal as result of a simple test

(3) No history of *past* relevant cardiac or respiratory disease (Six men with a history of pneumonia or pleurisy, uncomplicated and without sequelae, were included)

(4) No relevant *present* symptoms of cardiac or respiratory illness, i e no chronic cough, breathlessness or wheezing (Men subject to "chest colds" (with or without wheezing) were included if these were less than three a year)

(5) No physical signs of relevant cardiac or respiratory disease (Men with possible hypertension were included)

The physical and radiological measurements of the subjects examined are summarized in Table I (The means and standard deviations in this and subsequent tables, and the coefficients of correlation of Table III, were obtained by treating the data as individual ungrouped items) The more important frequency distributions of the measurements are shown in the Appendix

TABLE I PHYSICAL AND RADIOLOGICAL CHARACTERISTICS OF THF SIXTY-SIX NORMAL MALES

Characteristic	Aged 39 and under (39 subjects) Mean	Aged 40 and over (27 subjects) Mean	Significance of difference between means*	All ages Mean	All ages S D †	All ages C V ‡
Age last birthday (years)	31 7	47 8	—	38 3	9 6	25 1
Standing height (in)	66 1	65 5	1 0	65 8	2 3	3 5
Stem height (in)	35 3	34 6	2 1	35 0	1 3	3 8
Weight (lb)	139 7	145 3	1 2	142 0	18 8	13 2
Surface area (sq m)	1 70	1 73	1 0	1 71	0 13	7 5
Normal total basal calories per day (tens)	1610	1570	1 3	1590	124	7 8
Chest circumference (in)	36 0	36 8	1 4	36 3	2 3	6 3
Chest circumference expansion (in)	2 03	1 81	1 5	1 94	0 58	29 9
Area of lung fields (sq in)	94 4	91 8	1 2	93 3	8 9	9 5
Radiological chest volume (cu in)	793	816	0 9	803	109	13 6
Radiological chest height/width × 100	87 6	89 0	0 7	88 2	8 3	9 4

* Ratio of difference between means of the two age groups standard error of difference

† Standard deviation ‡ Coefficient of variation (percentage)

For statistical terminology see Hill (1937)

The mean age, 38 3 years, and the age distribution indicate that the sample is representative of the span of working life The mean height, weight, surface area and chest circumferential expansion are lower than would be expected in population groups in certain other parts of Great Britain, these differences being probably due to the Welsh racial characteristics

The commonly observed effects of increasing age are seen Thus weight appears to increase and height slightly to decrease Chest circumferential expansion also decreases, an observation in accordance with a common clinical impression It must be emphasized, however, that age differences of these and of the other physical and radiological measurements are all slight, and that in only one instance—stem height—is the difference more than twice the standard error of the difference

4 RESULTS OF DETERMINATION OF LUNG VOLUME

In the general presentation of these results we have followed the arrangement adopted by the Rochester workers, whose papers are exemplary in their statistical completeness

In Table II are set out the values of the lung volume and its subdivisions Complete estimations were possible in sixty-four of the sixty-six normal subjects In the other two, determination of functional residual air was impossible owing to inability to co-operate sufficiently, although the vital capacity was satisfactorily recorded and is therefore included in the table We shall subsequently discuss these results in comparison with the results obtained by previous workers, but in the meantime it may be noted that in general our mean absolute values of total lung volume and

TABLE II DETERMINATION OF LUNG VOLUME SUBDIVISIONS IN SIXTY-FOUR NORMAL MALES

Lung volume subdivision	Aged 39 and under (38 subjects) Mean	Aged 40 and over (26 subjects) Mean	Significance of difference between means	All ages Mean	All ages S D	All ages C V
		Absolute values (litres)				
Total lung volume	5 51	5 28	1 1	5 42	0 84	15 5
Vital capacity	3 98*	3 54†	3 1	3 80‡	0 55	14 5
Reserve air	1 39	1 22	2 3	1 32	0 30	22 9
Functional residual air	2 92	2 92	0 0	2 92	0 60	20 6
Residual air	1 52	1 70	1 5	1 59	0 50	31 6
		Relative values (percentage of total lung volume)				
Total lung volume	100 0	100 0	—	100 0	—	—
Vital capacity	72 9§	68 3§	—	71 0§	—	—
Reserve air	25 4	23 4	1 5	24 6	5 5	22 4
Functional residual air	52 6	55 3	1 7	53 7	6 3	11 7
Residual air	27 1	31 7	2 7	29 0	6 6	22 8
		Relative values (percentage of vital capacity)				
Total lung volume	—	—	—	—	—	—
Vital capacity	100 0	100 0	—	100 0	—	—
Reserve air	35 1	34 5	0 3	34 9	7 0	20 1
Functional residual air	-	—	—	—	—	—
Residual air	—	—	—	—	—	—

* Thirty nine subjects

† Twenty seven subjects

‡ Sixty six subjects for sixty four subjects the figure is 3 81

§ Obtained by subtracting residual air from 100

For other explanatory notes, see footnote to Table I vital capacity are lower than those of most of the previous series of normal males, and that this is probably largely the result of the small stature of the present subjects

All the tabulated absolute values show large coefficients of variation, probably due, to a great extent, to variations of individual build On account of these wide variations previous workers have preferred the use of *relative* values for purposes of comparison, i e total lung volume and vital capacity in terms of some physical and radiological measurement (see later, § 5), and the remaining subdivisions (residual air, reserve air, etc) expressed as percentages of the total lung volume or vital capacity It will be noted that the coefficients of variation for functional residual air and residual air in the present series are considerably reduced by this procedure, making these relative values more useful for comparison of pathological lung conditions with the normal

The main differences between subjects over and under 40 years of age are decreases in the total lung volume and in vital capacity, by 0 23 and 0 44 l respectively, a decrease in the absolute and relative values of reserve air, and an increase in the relative values of functional residual air and residual air The differences in absolute vital capacity and relative residual air are about three times their standard errors and therefore highly significant, the difference in absolute reserve air is about twice its standard error and is therefore just significant, while, since the other differences are less than twice their standard errors, they are no more than suggestive

These changes, which are similar to those described by Kaltreider, Fray and Hyde (1937, 1938), could be accounted for by

(*a*) alterations in the shape of the spine and thoracic cage with age,

(*b*) the latent development of emphysematous changes in the lung itself, and

(*c*) differences in the mean height of the two age groups

Studies on disease have shown that in emphysema particularly there occur characteristic changes in the functional residual air and residual air percentages In a case of emphysema that is recognizable clinically the upward shift in these values is very gross, as, for example

	Relative value (percentage of total lung volume)	
	Normal	Emphysema
Functional residual air	54	70
Residual air	29	50

It seems probable that shifts in these relative values of lung volume subdivisions will reveal the presence of emphysema at an earlier stage than will any other known method Conversely, since there is no other method of diagnosing early or latent emphysema, it may be expected that a few such cases will be encountered in any survey of the adult male population at all ages It will be noted from the Appendix that two such possible cases have been included, one with a functional residual air of 70 % and the other with a residual air of 46 % Although both these values are more than twice the standard deviation from the corresponding means, they are not excluded from the present series since they satisfied the criteria of normality already given, and since no precise dividing line between normal and abnormal ranges of values is at present available

5 CORRELATION OF TOTAL LUNG VOLUME AND VITAL CAPACITY WITH PHYSICAL AND RADIOLOGICAL MEASUREMENTS

In considering the absolute values for the lung volume and its subdivisions it was shown that the figures obtained have a wide variation, and it is therefore difficult to use directly these values in a survey of the alterations produced by disease The only figures in the preceding section which are of real practical importance in this connexion are the relative percentage values of residual and functional residual air The vital capacity may also be given as a percentage of the total lung volume, but this percentage is merely the converse of the relative residual air Both the vital capacity and the total lung volume are, however, more usefully expressed as a percentage of their normal values as predicted from independent physical or radiological measurements Following the Rochester workers, we have studied the degrees of correlation of the total lung volume and the vital capacity with certain readily obtainable measurements made on the same normal subjects Where high degrees of correlation were obtained regression equations suitable for the prediction of normal values have been calculated by the usual methods (see Hill 1937)

The correlation coefficients obtained are summarized in Table III Attention may be drawn to the following points

(1) High and significant correlation coefficients are found between total lung volume and standing height, stem height, surface area, normal total basal calories per day, area of lung fields and radiological chest volume, the last-mentioned coefficient being outstanding (0 80) A lower degree of correlation is found between total lung volume and both chest circum-

ference and body weight, while there is no significant correlation between this volume and either chest expansion or the chest height/width index.

(2) The correlations for vital capacity are broadly similar to those for total lung volume, except that the radiological chest volume is somewhat less correlated than is either standing or stem height or surface area, the coefficient for stem height being the best (0.78). It is interesting to note the smaller significance of the correlation between chest expansion and vital capacity (2.3 × standard error) than that obtaining with the actual circumference (4.3 × standard error). The absence of relationship between vital capacity and the index for shape of chest is also interesting, being contrary to the popular view that the broad muscular or hypersthenic chest is associated with a large vital capacity.

Table III. CORRELATION COEFFICIENTS BETWEEN PHYSICAL AND RADIOLOGICAL CHARACTERISTICS OF NORMAL MALES AND (i) TOTAL LUNG VOLUME, (ii) VITAL CAPACITY

Characteristics	(i) Correlation with total lung volume (64 subjects)	(ii) Correlation with vital capacity (66 subjects)
Standing height	+0.66	+0.70
Stem height	+0.66	+0.78
Weight	+0.55	+0.57
Surface area	+0.64	+0.67
Normal total basal calories per day	+0.63	+0.73
Chest circumference	+0.53	+0.51
Chest circumference expansion	+0.14	+0.28
Area of lung fields	+0.65	+0.59
Radiological chest volume	+0.80	+0.63
Radiological chest height/width × 100	+0.11	−0.14

When a sample of size n is drawn from a bivariate universe with zero correlation the standard error of an observed correlation will be $1/(n-1)^{\frac{1}{2}}$ = approximately 0.12 in the present case.

From consideration of these correlations, therefore, a number of physical and radiological measurements in the present series of normal males suggest themselves for the basis of prediction formulae both for normal total lung volume and for normal vital capacity. The choice was narrowed down by the following additional considerations.

(1) Normal total basal calories per day offers no advantage over the surface area, and, furthermore, it is not a measurement in general use.

(2) Chest circumference is indirectly taken into account by the radiological chest volume, while area of lung fields is actually used in the

computation of the latter Furthermore, in the present series radiological chest volume is more highly correlated with total lung volume and vital capacity than are either of these other measurements

(3) A multiple correlation calculated between standing height and weight on the one hand and total lung volume on the other hand ($R = +0\ 69$) shows only a slight increase over the simple correlation coefficient for height alone (+0 66), which latter is itself greater than that for weight alone (+0 55) Similar considerations hold for multiple correlations between standing height and weight and vital capacity ($R = +0\ 72$) in comparison with the coefficients for height (+0 70) and for weight (+0 57) alone The reason for the lack of usefulness of these multiple correlations is that the two independent variables, height and weight, are moderately well correlated with one another ($r = +0\ 60$)

(4) Surface area (another method of taking into account both height and weight) is no more highly correlated with total lung volume and vital capacity than is standing height

There thus remain three measurements to be considered as a basis for prediction formulae for total lung volume and vital capacity, namely, standing height, stem height and radiological chest volume

In order to determine whether standing height and radiological chest volume could be usefully combined, these variables were correlated with one another ($r = +0\ 66$) A multiple correlation was then calculated between standing height and radiological chest volume on the one hand and total lung volume on the other The resulting multiple correlation coefficient (+0 82) is hardly higher than the simple coefficient for radiological chest volume alone (+0 80), which latter is itself greater than that for standing height alone (+0 66) A similar multiple correlation was made between standing height and radiological chest volume and vital capacity The coefficient (+0 73) is only slightly larger than the simple coefficient for standing height alone (+0 70), this latter being itself greater than the coefficient for radiological chest volume alone (+0 63) It appears, therefore, that a regression formula based upon a combination of height and radiological chest volume is unlikely to be of sufficient value for predicting either normal total lung volume or normal vital capacity to justify the rather more complicated calculations required

In Table IV the regression equations separately relating standing height, stem height and radiological chest volume with total lung volume and vital capacity are shown, and in fig 1 the individual points are graphed to show their scatter about the regression lines

From the foregoing considerations it would seem that the best measurement for predicting normal total lung volume is the radiological chest volume, and for predicting normal vital capacity is either the standing or the stem height.

TABLE IV. CONSTANTS (p, q) IN REGRESSION EQUATIONS ($y = px + q$) RELATING CERTAIN PHYSICAL AND RADIOLOGICAL CHARACTERISTICS OF NORMAL MALES AND (I) TOTAL LUNG VOLUME, (II) VITAL CAPACITY (DERIVED FROM TABLES I–III)

(i) Total lung volume (in litres) (y)
(Standard deviation of y = 0.84 l.)

x	p	q	S.E.* (litres)
Standing height (in in.)	0.240	−10.42	0.63
Stem height (in in.)	0.417	− 9.17	0.63
Radiological chest volume (in cu. in.)	0.00615	+ 0.48	0.50

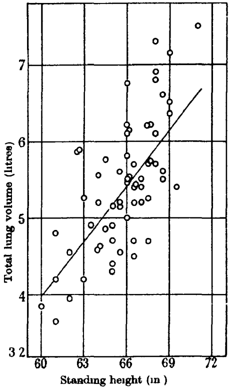
(ii) Vital capacity (in litres) (y)
(Standard deviation of y = 0.55 l.)

x	p	q	S.E.* (litres)
Standing height (in in.)	0.169	− 7.34	0.39
Stem height (in in.)	0.324	− 7.52	0.35
Radiological chest volume (in cu. in.)	0.00318	+ 1.25	0.43

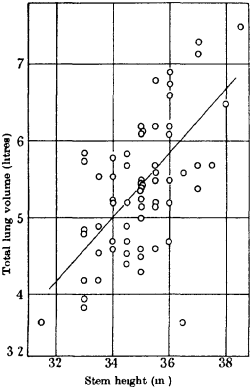
[*] Standard error of estimate of y, for comparison with its standard deviation.

The predicted normal value for total lung volume for each member of the present series of normal males was calculated by means of the regression formula relating total lung volume with radiological chest volume (Table IV), and the value actually observed was then expressed as a percentage of its predicted normal value. A similar procedure was followed for vital capacity, using the regression equation relating the latter with stem height. In this way two series of relative percentage values, one of total lung volume and the other of vital capacity, were derived. The results of this procedure are tabulated (Table V), and their frequency distributions are given in the Appendix. The mean of these relative values for the whole age group should clearly approximate to 100 %, though both vital capacity and total lung volume tend to diminish with increasing age, as was noted with the absolute values. Clearly, these age differences must be borne in mind in comparison with pathological conditions.

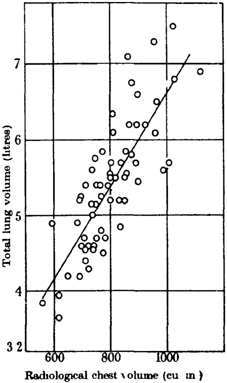
Even though the coefficients of variation of these relative values are reduced in comparison with the variations of the means of the absolute total lung volume and vital capacity, the considerable scatter remaining (see fig. 1) makes the use of the prediction formulae of very limited value for estimating the normality or abnormality of the observed findings in an



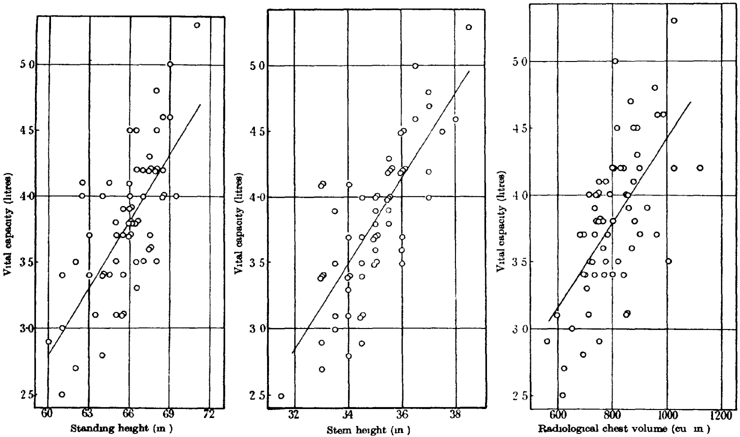
Equation of regression line $y = 0{\cdot}240x - 10\ 42$
Correlation coefficient $r = +0\ 66$



Equation of regression line $y = 0\ 417x - 9\ 17$
Correlation coefficient $r = +0\ 66$



Equation of regression line $y = 0{\cdot}00615x + 0{\cdot}48$
Correlation coefficient $r = +0{\cdot}80$



Equation of regression line $y = 0{\cdot}169x - 7\ 34$
Correlation coefficient $r = +0\ 70$

Equation of regression line $y = 0{\cdot}324x - 7\ 52$
Correlation coefficient $r = +0\ 78$

Equation of regression line $y = 0\ 00318x + 1\ 25$
Correlation coefficient $r = +0{\cdot}63$

Fig 1

individual case On the other hand, the mean and distribution of the relative values of a normal *group* may afford a useful comparison with corresponding values in groups of abnormal subjects

TABLE V RELATIVE VALUES OF TOTAL LUNG VOLUME AND VITAL CAPACITY, EXPRESSED AS PERCENTAGE OF PREDICTED NORMAL VALUES*

	Aged 39 and under		Aged 40 and over		All ages		
	Mean	S D	Mean	S D	Mean	S D	C V
Total lung volume	102 5 (38)	8 9	96 4 (26)	8 2	100 0 (64)	9 2	9 2
Vital capacity	102 0 (39)	10 1	96 4 (27)	8 4	99 7 (66)	9 8	9 9

* Normal values for total lung volume are predicted from the regression equation relating this variable to radiological chest volume Normal values for vital capacity are predicted from the regression equation relating this variable to stem height (see Table IV)

Bracketed figures show number of cases

For other explanatory notes, see footnote to Table I

Unless, however, the distribution of radiological chest volume or height of these abnormal groups differs from that of the normal groups, relative values of vital capacity or of total lung volume offer, of course, little advantage for comparison over the absolute values

6 THE EFFECTS OF OBESITY UPON THE LUNG VOLUME AND ITS SUBDIVISIONS

At an early stage of the work it was noted that obese subjects tended to give low reserve air values For the purpose of further investigation, all subjects were classed, by clinical assessment of the amount of subcutaneous adipose tissue, into obese and non-obese groups The physical and radiological characteristics and lung volume determinations of these two groups are shown in Table VI

As might be expected, the mean age, weight and surface area of the obese group are increased, while the height is not different from that of the non-obese Furthermore, the radiological chest volume is greater, and the chest height/width ratio smaller, than in the non-obese

When age is allowed for, by use of expected means, the absolute total lung volume and vital capacity are slightly greater in the obese group Since, as has already been pointed out, the normal total lung volume is closely correlated with the radiological chest volume, allowance for

TABLE VI COMPARISON OF (*a*) PHYSICAL AND RADIOLOGICAL CHARACTERISTICS AND (*b*) LUNG VOLUME DETERMINATIONS, IN OBESE AND NON-OBESE MEMBERS OF THE GROUP OF NORMAL MALES

(*a*) Physical and radiological characteristics

Characteristic	Non obese (49 subjects) Observed mean	Obese (17 subjects) Observed mean	Obese (17 subjects) Expected* mean
Age last birthday† (years)	36 4	43 7	—
Standing height (in)	65 8	65 9	65 7
Stem height (in)	35 0	34 9	34 9
Weight (lb)	135 9	159 7	136 6
Surface area (sq m)	1 68	1 80	1 68
Radiological chest volume (cu in)	787	848	795
Radiological chest height/width × 100	90 2	82 5	92 3

(*b*) Lung volume determinations

Lung volume subdivision	Non obese (47 subjects) Observed mean	Obese (17 subjects) Observed mean	Obese (17 subjects) Expected* mean
Absolute values (litres)			
Total lung volume	5 33	5 66	5 27
Vital capacity	3 78‡	3 85	3 63
Relative values (percentage of predicted normal values§)			
Total lung volume	100 3	99 1	98 6
Vital capacity	99 0‡	101 7	96 1
Relative values (percentage of total lung volume)			
Reserve air	26 4	19 7	26 3
Functional residual air	54 5	51 4	56 2
Residual air	28 0	31 6	29 9
Relative values (percentage of vital capacity)			
Reserve air	37 0	28 9	37 9

* Expected for a non obese population of similar age distribution (Based upon the means of different ages (20–29, 30–39, 40–64 years) in the non obese group, and the numbers at the same ages in the obese group)

† Range of age in the obese group 30–62 years, and in the non obese group 19–63 years

‡ Forty nine subjects

§ Predicted from regression equations relating total lung volume to radiological chest volume, and vital capacity to stem height (see Table IV).

variations in the latter should reduce the differences in the total lung volume in the two groups Indeed, these differences practically disappear when the total lung volume is expressed as a percentage of its normal value, predicted from the radiological chest volume When, similarly, vital capacity is expressed as a percentage of its normal value predicted from stem height, the slight difference in its value between the obese and non-obese groups remains since the height in the two groups is the same This latter difference is possibly accounted for by a slight partial correlation existing between vital capacity and weight when the influence of radiological chest volume is excluded

The most interesting points in the table are that the obese group has a substantially decreased relative reserve air (expressed as percentage of total lung volume) and a decreased relative functional residual air, while, when age differences are allowed for, the relative residual air is almost the same The deduction is that the loss in the reserve air corresponds to a gain in the complementary air this is confirmed by the substantially lower relative value for the reserve air of the obese group when expressed as percentage of the vital capacity

Hurtado and Fray (1933*a*) pointed out an association between the radiological chest height/width ratio and the (absolute) reserve air in their normal subjects, finding a significant correlation coefficient of +0 41 (probable error 0 08) In the present series (obese and non-obese together) a just significant correlation coefficient of +0 28 (standard error 0 12) was also found between these two variables Since, as shown in Table V, the obese group has, on the average, a lower chest height/width index than the non-obese, it is possible that part of the differences in reserve air noted are due to the coincident differences in the radiological shape of the chest

7 DISCUSSION

(*a*) *Comparison with results of previous workers*

(i) *Lung volume determinations* While the literature on the vital capacity of normal males is large, few previous investigators, besides Hurtado and his co-workers, have made complete estimations of the total lung volume, together with its subdivisions, in the same series of persons For purposes of comparison with the present series the results of these investigators are set out in Table VII, A and B Both series of males studied by Hurtado and his collaborators, one composed of young adults (aged 18–30 years), and the other of older subjects (aged 38–63 years), are shown, the majority of their figures being those recalculated in their 1938 paper, taking into account a correction for nitrogen diffusion from the blood The figures of "other authors" are those collected by the Rochester workers from the papers of Anthony, Binger, Lundsgaard and Van Slyke, and Lundsgaard and Schierbeck Previous figures for females are also included as a matter of general interest, though these are not discussed here.

The most important fact to be kept in mind in comparing these various results is the small stature of the subjects in the present series The following points arising from variations in technique and choice of subjects must also be considered

(1) Although we have pointed out the possibility of error in the Christie technique at values of functional residual air of over 3 5 l, it would appear that the determinations of Hurtado and his co-workers have not been appreciably influenced by this source of difficulty

(2) Previous authors, other than the Rochester workers, measured the mid-capacity (functional residual air) from the mid tidal air level instead of from the resting respiratory level

(3) Hurtado's X ray films were obtained at a distance of 6 ft, while those in our series were taken at 4½ ft This would increase the area of the lung fields in our films by approximately 4 % above that in 6 ft films

(4) The recumbent position in which Hurtado's determinations were made lowers the total lung volume by 9–10 % on the average (Hurtado and Fray 1933*b*, Kaltreider, Fray and Hyde 1938), and also produces a considerable shift in the resting respiratory level, with an increase in the complementary air and a decrease in functional residual air The recumbent position also lowers the radiological chest volume by about 8 %

(5) The two series of males studied by Hurtado and his co-workers were taken from different portions of the population The subjects of the younger series were college students, while those of the older group were mainly artisans The difference in the height of the two series—2 3 in —is probably greater than can be accounted for by age, while the similarity in the weight, bearing in mind the difference of age, also suggests lack of homogeneity from one group to the other

The small stature of the present series is shown by comparing the first two columns of Table VII Correspondingly the absolute values of the total lung volume and vital capacity are smaller than those of both series of males investigated in the sitting posture by Hurtado and his co-workers, and are also smaller than those given for males in a similar posture by other authors When the present relative values of the lung volume subdivisions are compared with those given for the sitting posture by Hurtado and his collaborators and by other workers, a fair degree of similarity is seen on the other hand, when the comparison is made with the recumbent series of the Rochester workers, considerable differences are shown

It thus appears that the differences in the lung volume determinations in these various series can to a large extent be accounted for by variations in stature and posture

TABLE VII COMPARISON OF PRESENT RESULTS WITH THOSE OF PREVIOUS WORKERS

A Physical and radiological characteristics (mean)

	Males						Females	
	Present series	Hurtado and co workers*				Other authors†	Hurtado and co workers*	Other authors†
Characteristic	(66 subjects) (sitting)	(50 subjects) (recumbent) series 1	(50 subjects) (recumbent) series 2	(10 subjects) (sitting) series 1	(10 subjects) (sitting) series 2	(46 subjects) (standing or sitting)	(50 subjects) (recumbent)	(22 subjects) (sitting)
Age (years)	38 3	22 9	48 2	23 0	44 5	—	23 1	—
Standing height (in)	65 8	69 4	67 1	—	—	—	64 3	—
Stem height (in)	35 0	—	—	—	—	—	—	—
Weight (lb)	142 0	159 8	156 0	—	—	—	125 8	—
Surface area (sq m)	1 71	1 88	1 82	—	—	—	1 60	—
Chest circumference expansion (in)	1 94	3 6	—	—	—	—	—	—
Area of lung fields (sq in)	93 3	106 2	101 2	—	—	—	—	—
Radiological chest volume (cu in)	803	905	921	—	—	—	624	—

B Lung volume determinations (mean)

	Males						Females	
	Present series	Hurtado and co-workers*				Other authors†	Hurtado and co workers*	Other authors†
Lung volume subdivision	(64 subjects) (sitting)	(50 subjects) (recumbent) series 1	(50 subjects) (recumbent) series 2	(10 subjects) (sitting) series 1	(10 subjects) (sitting) series 2	(46 subjects) (standing or sitting)	(50 subjects) (recumbent)	(22 subjects) (sitting)
Absolute values (litres)								
Total lung volume	5·42	5 97	5 37	6 64	5 92	5 98	4 24	4 41
Vital capacity	3 80‡	4 78	4 07	4 99	4 30	4 46	3 14	3 18
Functional residual air	2 92	2 18	2 00	3 33	2 92	3 70§	1 82	2 76§
Residual air	1 59	1 19	1 30	1 65	1 62	1 50	1 10	1 23

Relative values (percentage of total lung volume)								
Vital capacity	71·0	80·2	75·5	75·4	72·7	75·1	74·1	72·0
Functional residual air	53·7	36·2	37·7	49·9	49·6	60·7§	43·0	62·5§
Residual air	29·0	19·8	24·5	24·5	27·3	25·3	25·9	28·2

C Correlation coefficients

Physical and radiological characteristics	Correlation with total lung volume				Correlation with vital capacity			
	Present series	Hurtado and co workers*			Present series	Hurtado and co-workers*		
	(64 males)	(50 males) series 1	(50 males) series 2	(50 females)	(66 males)	(50 males) series 1	(50 males) series 2	(50 females)
Standing height	+0·66	+0·48	+0·62	+0·66	+0·70	+0·55	+0·64	+0·51
Stem height	+0·66	—	—	—	+0·78	—	—	—
Weight	+0·55	+0·01	+0·36	+0·18	+0·57	+0·14	+0·31	+0·26
Surface area	+0·64	+0·15	+0·44	+0·36	+0·67	+0·28	+0·45	+0·34
Chest circumference	+0·53	+0·14	—	—	+0·51	+0·16	—	—
Area of lung fields	+0·65	+0·55	+0·79	+0·63	+0·59	+0·68	+0·68	+0·60
Radiological chest volume	+0·80	+0·61	+0·85	+0·63	+0·63	+0·72	+0·73	+0·71

[*] **Hurtado and Boller** (1933), **Hurtado and Fray** (1933*a*, 1933*b*), **Hurtado, Fray, Kaltreider and Brooks** (1934), **Hurtado, Kaltreider, Fray, Brooks and McCann** (1934), **Kaltreider, Fray and Hyde** (1938) Many of the original figures were corrected by slight amounts in the last-named paper

[†] **Anthony, Binger, Lundsgaard and Van Slyke, Lundsgaard and Schierbeck** (Collected and tabulated by **Hurtado and Boller** (1933) and **Hurtado, Fray, Kaltreider and Brooks** (1934))

[‡] Sixty-six subjects.

[§] Functional residual air plus half tidal air

(ii) *Correlations of total lung volume and vital capacity with physical and radiological measurements* Few previous workers have related total lung volume to body measurements in order to obtain formulae for predicting normal values in the individual Lundsgaard and Van Slyke (1918) considered that total lung volume could be expressed as a simple function of the chest volume measured externally Hurtado and his co-workers, in their more extensive survey, were, however, unable to confirm this finding The latter workers were apparently the first to include radiological measurements among the characteristics investigated Since, as we have done, they used the same subjects as a basis for ascertaining the relationship to various physical and radiological measurements both of vital capacity and of total lung volume, it is convenient to discuss all their correlations together (i e those of vital capacity as well as those of total lung volume) in comparison with ours, and then to consider the previous large literature upon the prediction of vital capacity alone

The present correlations, both of total lung volume and of vital capacity, agree with those of the two series of males studied by the Rochester workers, except in the following points (see Table VII c)

(1) Our correlations, both of vital capacity and of total lung volume, with weight, surface area and chest circumference are all highly or moderately significant

(2) The highest of all our correlations is that between radiological chest volume and total lung volume (+0 80) While this was also the case in the series of older males of Hurtado and his co-workers (+0 85), in their younger series the radiological chest volume was somewhat more highly correlated with vital capacity (+0 72) than with total lung volume (+0 61)

(3) Our highest correlation for vital capacity is that with stem height (+0 78), while standing height gives a somewhat higher degree of correlation with vital capacity (+0 70) than was obtained by Hurtado and his co-workers in their two series (+0 55 and +0 64)

It is interesting to note that Hurtado and his co-workers obtained, in general, higher correlations between radiological and physical measurements and the total lung volume and vital capacity in their older group of males than in their younger series

From their correlation coefficients Hurtado and his co-workers concluded that the radiological chest volume surpassed any external measurement both for predicting normal total lung volume and normal vital capacity We are in agreement with these workers as regards total lung volume, but not as regards vital capacity, since the present results suggest that for predicting normal vital capacity the ordinary physical characteristics, standing or stem height, may be more valuable than are radiological measurements, while surface area also would appear to be satisfactory for this purpose In this connexion it may be noted, in the Rochester series, that although vital capacity was less correlated with standing height than it was with radiological chest volume, it showed quite a substantial correlation with the former variable, though the authors do not stress this point vital capacity was not, however, well correlated with surface area

For predicting the normal vital capacity in their series of young adult males, the formula used by Hurtado and his co-workers was the linear regression equation connecting vital capacity and radiological chest volume The prediction formula for normal total lung volume was not separately derived, but was based upon their mean ratio of total lung volume to vital capacity, the predicted normal vital capacity in each case being multiplied by this constant Hurtado and his co-workers thus derived this formula for relating total lung volume to radiological chest volume indirectly, and it is, therefore, not strictly comparable with our regression equation, which was obtained directly

In their series of older males the Rochester workers (Kaltreider, Fray and Hyde 1938) proceeded in the opposite manner, obtaining the prediction formula for normal total lung volume directly from the regression equation connecting total lung volume with radiological chest volume, and obtaining the prediction formula for normal vital capacity indirectly, by use of the mean ratio of vital capacity to total lung volume This formula for predicting normal total lung volume would, therefore, be more comparable with our regression equation for relating total lung volume to radiological chest volume were it not for the difference in age distribution of the subjects from which it was derived (Hurtado and his co-workers also give an alternative formula for predicting normal vital capacity, which was obtained directly)

Having compared our correlations of total lung volume and vital capacity with those of the Rochester workers, it remains briefly to consider our vital capacity correlations in the light of earlier investigations into this part of the subject In contrast to the extreme paucity of work upon total lung volume in relation to body measurements in normal males, the literature upon vital capacity is large, and only some of the more representative papers will be quoted, attention being paid to two points first, the physical measurements found to be most closely related to vital capacity, and, second, the nature of this relationship (For the main literature the reader is referred to Myers (1925) and Moncrieff (1934))

In his classical work Hutchinson (1846) provided evidence that vital capacity could be expressed as a simple function of standing height, the volume of air increasing regularly with the latter He stated that 8 extra cu in of air at 60° F were expelled for each extra inch of height between 5 and 6 ft The relationship with weight was found to be less uniform, while none was noted with chest circumference

Dreyer (1919) was unable to support Hutchinson's view of a simple linear relationship between vital capacity and height He advocated separate prediction formulae of the form, $y = ax^b$, where a and b are constants, for height (stem preferred to standing height), weight and chest circumference, these, however, resolved into a simple relationship of the form, $y = ax$, when surface area was the independent variable West (1920) also expressed vital capacity as a linear function of surface area, giving the formula, y (litres) $= 2\ 5x$ (square metres) He believed that this combination of height and weight offered the best basis for prediction, and that height itself was the next best measurement

Cripps, Greenwood and Newbold (1923), in an extensive study of 950 Royal Air Force candidates passed as fit, examined a variety of possible relationships between vital capacity and standing height, stem height, weight, chest circumference and age They concluded that multiple linear regression equations involving several of these independent variables were a rather better method of predicting normal vital capacity than were Dreyer's formulae However, even when four independent variables (standing height, stem height, weight and chest circumference) were combined, the multiple correlation with vital capacity (+ 0 64) was only little higher than the highest of the simple correlation coefficients The latter were, in descending order of magnitude, + 0 59 (standing height), + 0 54 (stem height), + 0 49 (weight) and + 0 39 (chest circumference) The finding that height was the best of these simple correlations was in agreement with Hutchinson's finding of a linear relationship between vital capacity and this variable The authors pointed out a further similarity in that their formula relating standing height to vital capacity gave a figure of 8 87 cu in , corresponding to Hutchinson's figure of 8 cu in (see also Cripps 1924)

From the above and other literature consulted, height (standing or stem) appears to be the most favoured external physical measurement for predicting normal vital capacity Surface area is a close second, while weight and chest circumference are less satisfactory With these conclusions the present investigation is in complete agreement While stem height has here been chosen for later comparison with pathological cases, because the value of its correlation was higher in this series than was that of standing height, the difference between the two is not significant It may be noted that our formula relating standing height to vital capacity gives a constant of 10 3 cu in , corresponding to Cripps, Greenwood and Newbold's 8 9 cu in , and to Hutchinson's 8 cu in

(b) *Applicability of lung volume determinations*

It is to be noted that the present results have been worked out on a selected population of small stature Consequently, the standards obtained are not necessarily applicable outside the range of physical measurements covered by this group

Generally speaking, the wide scatter of the absolute values of lung volume determinations detracts from their usefulness in the individual Even by the use of values relative to the total lung volume or to a normal value predicted from certain physical and radiological measurements, the reduction in scatter is insufficient to make individual results valuable except when the deviations are marked On an adequate number of pathological cases, however, statistical analysis of absolute and relative values, particularly the latter, should be of use for comparison with normal controls It is important that the physical characteristics of such pathological cases should lie within the range covered by the controls Since the most suitable measurements for predicting normal total lung volume and vital capacity are radiological chest volume and stem or standing height, respectively, it is to these characteristics that most attention should be paid

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SUMMARY

1 Determinations of the total lung volume and its subdivisions have been made in the sitting position in sixty-four men, aged 19–63 years, selected from normal members of a mining population in South Wales The more important physical and radiological measurements of the group are tabulated The estimation of lung volume was made by a modification of Christie's method

2 The findings of previous workers who observed a wide variation in the absolute values of the total lung volume and its subdivisions have been confirmed This variation takes place within a narrower range if the subdivisions are expressed as percentages of the total lung volume

3 In advancing age the total lung volume and vital capacity diminish slightly, with an increase in the relative value of the residual air expressed as a percentage of the total lung volume

4 Correlation coefficients have been calculated between total lung volume and vital capacity, on the one hand, and various physical and radiological measurements, on the other The highest degrees of correlation in this series are those existing between total lung volume and "radiological chest volume" (+0 80), as measured by the method of Hurtado and his co-workers, and between vital capacity and stem height (+0 78) High or moderate degrees of correlation are, moreover, found with various common physical measurements, such as standing height and surface area From the statistical data regression formulae for the prediction of normal total lung volume and normal vital capacity in individual cases have been determined The limitations of such formulae in the demonstration of abnormality are pointed out

5 It has been found that obesity is associated with a considerable reduction in the value of the reserve air, with a corresponding gain in the complementary air Part of this association may be due to the broad or hypersthenic type of chest frequently found in obese subjects

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APPENDIX A. FREQUENCY DISTRIBUTIONS OF MORE IMPORTANT PHYSICAL AND RADIOLOGICAL MEASUREMENTS OF NORMAL MALES EXAMINED

Age		Standing height				Stem height				Weight			
Age last birthday (years)	No (Range 19–63 years)	Height (in)	Aged 39 and under (Range 60·0–71·0 in)	Aged 40 and over (Range 61·0–68·5 in)	All ages (Range 60·0–71·0 in)	Height (in)	Aged 39 and under (Range 33·0–38·5 in)	Aged 40 and over (Range 31·5–36·5 in)	All ages (Range 31·5–38·5 in)	Weight (lb)	Aged 39 and under (Range 100–192 lb)	Aged 40 and over (Range 115–188 lb)	All ages (Range 100–192 lb)
15–	1	60–	1	—	1	31–	—	1	1	100–	1	—	1
20–	2	61–	1	2	3	32–	—	—	—	110–	3	2	5
25–	8	62–	4	—	4	33–	7	4	11	120–	10	5	15
30–	15	63–	1	2	3	34–	4	10	14	130–	9	3	12
35–	13	64–	4	2	6	35–	15	8	23	140–	4	5	9
40–	10	65–	2	8	10	36–	6	4	10	150–	6	7	13
45–	9	66–	9	7	16	37–	5	—	5	160–	4	3	7
50–	4	67–	7	2	9	38–38·75	2	—	2	170–	1	1	2
55–	2	68–	5	4	9					180–	—	1	1
60–64	2	69–	4	—	4					190–199·5	1	—	1
		70–	—	—	—								
		71–71·75	1	—	1								
Total	66		39	27	66		39	27	66		39	27	66

APPENDIX A (*continued*)

Surface area				Area of lung fields				Radiological chest volume			
Surface area (sq m)	Aged 39 and under (Range 1 40– 2 00 sq m)	Aged 40 and over (Range 1 50– 2 00 sq m)	All ages (Range 1 40– 2 00 sq m)	Area (sq in)	Aged 39 and under (Range 74 5– 113 8 sq in)	Aged 40 and over (Range 69 5– 106 8 sq in)	All ages (Range 69·5– 113 8 sq in)	Volume (litres)	Aged 39 and under (Range 559– 1027 litres)	Aged 40 and over (Range 595– 1121 litres)	All ages (Range 559– 1121 litres)
1 4–	2	—	2	65–	—	1	1	550–	1	1	2
1 5–	4	4	8	70–	1	1	2	600–	2	1	3
1 6–	11	6	17	75–	3	1	4	650–	3	2	5
1 7–	9	5	14	80–	—	1	1	700–	9	5	14
1 8–	11	10	21	85–	5	7	12	750–	8	3	11
1 9–	1	1	2	90–	11	5	16	800–	5	4	9
2 0–2 075	1	1	2	95–	10	5	15	850–	7	6	13
				100–	3	5	8	900–	—	1	1
				105–	4	1	5	950–	2	2	4
				110–114 95	2	—	2	1000–	2	1	3
								1050–	—	—	—
								1100–1149 5	—	1	1
	39	27	66		39	27	66		39	27	66

APPENDIX B FREQUENCY DISTRIBUTIONS OF THE MORE IMPORTANT LUNG VOLUME DETERMINATIONS OF NORMAL MALES EXAMINED

Total lung volume				Vital capacity				Functional residual air (percentage of total lung volume)			
Total lung volume (litres)	Aged 39 and under (Range 3·85–7·50 l)	Aged 40 and over (Range 3·65–6·90 l)	All ages (Range 3·65–7·50 l)	Vital capacity (litres)	Aged 39 and under (Range 2·5–5·31 l)	Aged 40 and over (Range 2·7–4·61 l)	All ages (Range 2·5–5·31 l)	Functional residual air (%)	Aged 39 and under (Range 38·5–67·0%)	Aged 40 and over (Range 39·0–70·0%)	All ages (Range 38·5–70·0%)
3·6–	2	1	3	2·2–	—	1	1	36–	1	1	2
4·0–	2	1	3	2·6–	3	1	4	40–	1	—	1
4·4–	6	4	10	3·0–	1	5	6	44–	7	3	10
4·8–	2	5	7	3·4–	7	12	19	48–	5	4	9
5·2–	8	8	16	3·8–	13	6	19	52–	13	6	19
5·6–	8	2	10	4·2–	10	1	11	56–	8	7	15
6·0–	3	4	7	4·6–	3	1	4	60–	2	1	3
6·4–	3	—	3	5·0–5·35	2	—	2	64–	1	3	4
6·8–	2	1	3					68–71·95	0	1	1
7·2–7·575	2	—	2								
Total	38	26	64		39	27	66		38	26	64

APPENDIX B (*continued*)

Residual air (%)	Residual air (percentage of total lung volume): Aged 39 and under (Range 13·0–46·0%)	Aged 40 and over (Range 18·0–40·5%)	All ages (Range 13·0–46·0%)
12–	2	—	2
16–	3	2	5
20–	6	—	6
24–	7	4	11
28–	12	8	20
32–	6	2	8
36–	1	9	10
40–	—	1	1
44–47·95	1	—	1
Total	38	26	64

Total lung volume (%)	Total lung volume (percentage of predicted normal value*): Aged 39 and under (Range 85·7–123·5%)	Aged 40 and over (Range 85·5–118·4%)	All ages (Range 85·5–123·5%)
85–	3	5	8
90–	6	7	13
95–	6	5	11
100–	9	6	15
105–	3	1	4
110–	7	1	8
115–	3	1	4
120–124·95	1	—	1
	38	26	64

Vital capacity (%)	Vital capacity (percentage of predicted normal value†): Aged 39 and under (Range 80·0–129·3%)	Aged 40 and over (Range 79·2–117·1%)	All ages (Range 79·2–129·3%)
75–	—	1	1
80–	1	3	4
85–	3	2	5
90–	5	5	10
95–	8	7	15
100–	6	4	10
105–	12	4	16
110–	—	—	—
115–	2	1	3
120–	—	—	—
125–129·95	2	—	2
	39	27	66

* Predicted from regression equation relating total lung volume to radiological chest volume (see Table IV)

† Predicted from regression equation relating vital capacity to stem height (see Table IV)

576 858 8 578 089 82

Micrurgical studies on virus-infected plants

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[Plates 28]

Introduction

Micromanipulative methods have been used very seldom for the study of plant cells chiefly because glass instruments which are the only ones available are usually either too brittle or too flexible to penetrate the tough cell walls A few plant cells (e g the staminal hairs of *Tradescantia*) are suitable for micrurgical studies, but these are of little use for virus work Eleven different viruses were inoculated into each of three species of *Tradescantia* without any infections resulting

By the use of specially shaped needles it has been found possible to work with many of the cells of Solanaceous plants The cutinized hair cells of *Solanum nodiflorum*, which can rarely be penetrated through the side walls, can be successfully operated on if the protoplast is approached through the transverse septum For many operations these cells are particularly favourable, as diffusion of the mounting medium into the cell is very gradual, most of the large surface being protected by the thickened wall Livingstone and Duggar (1934) have compared the virus content of different parts of the infected tobacco cell and of cells containing and cells devoid of inclusions In this paper various properties of infected cells, including their virus content, will be described

Description

The pH *of the cell contents*

Many determinations have been made by indicators of the *p*H of the cell contents, but every method described is open to the grave criticism that it involves considerable injury to the cell and a lapse of time before any reading is obtained Injury to the cell is known to increase the hydrogen-ion concentration A method suggested by Professor Péterfi, although not entirely free from the possibility of error, is the most accurate yet defined A little dry indicator is dropped on to a spot of vaseline, from which it is picked up on the tip of a micro-needle The tissue to be examined is arranged in a hanging drop and the cell is punctured with the needle bearing the dye The indicator dissolves at once in the cell sap and the colour is immediately noted Indicators which change their colour and not merely their depth of colour are used A possible source of error lies in the wounding of the cell, but this error should not be great as the wound is small and the reading is taken very quickly

Various tissues from healthy plants of *Solanum nodiflorum*, from the same species infected with aucuba mosaic virus (Henderson Smith 1928), from healthy *Hyoscyamus niger* and from *H niger* infected with *Hyoscyamus* virus 3 (Hamilton 1932) were examined The object was primarily to compare virus-free and virus-diseased tissue but the following results accrued

(1) The *p*H of the contents of the hairs of the two species, in both healthy and diseased plants was approximately the same (*p*H 6 8)

(2) The contents of the epidermis and the palisade tissue were in all cases slightly less acidic than the hair cells, the *p*H lying between 6 8 and 7 2

(3) No differences were observed in the hydrogen-ion concentration between healthy and diseased plants in any type of cells

The stability of the intracellular inclusions

The amorphous inclusion bodies The vacuolated amoeboid inclusions of tobacco mosaic disease have been described as very fragile structures which break down on contact with the micro-needle but withstand considerable disturbance in the cytoplasm in their immediate vicinity (Livingstone and Duggar 1934) In aucuba mosaic disease, inclusions are produced by the gradual aggregation and fusion of minute particles which circulate in the cytoplasmic stream a few days after inoculation (Sheffield 1931) These inclusions are similarly frail, very much more so than is the cell nucleus Slight mechanical pressure on the outside of the cell destroys the inclusion immediately, although the cell may not be sufficiently crushed for any other injury to be visible Puncturing the inclusion with a very fine needle causes its immediate disintegration The three pictures 1 *a*, *b* and *c* (Plate 28) were obtained by working with a Leitz Makam camera attached to the microscope, the operations being watched through the side observation tube of the camera After the first picture (1*a*) was taken the plate was exchanged and the dark slide removed in readiness for a second exposure, so that fig 1*b* could be taken with the shortest possible delay after the inclusion was punctured In the first photograph of the series, the needle is seen to have penetrated the transverse septum and to have passed well into the vacuole The inclusion, not having been touched, is quite unaffected Then the inclusion body was punctured and the needle rapidly withdrawn 15 sec after the puncture the inclusion is seen to have changed its consistency and to have formed a mass of clear bubbles amongst which a few granules are visible (fig 1*b*) These granules are morphologically similar to those which went to form the body After a further 10 min the bubbles have disappeared and a few scattered granules only remain (fig 1*c*) Figs 2 and 3 (Plate 28) are comparable to fig 1*b* showing stages in a similar disintegration following injury of the inclusion body On injury an inclusion may disintegrate with the formation of a few granules without first forming a vesicle If two inclusions are present in a cell and one only is pierced, that one disintegrates, the other remaining quite unchanged

Despite their fragility these inclusions can, if certain precautions are taken, be removed intact from the cells containing them (fig 4, Plate 28) It is necessary, besides selecting a cell which can be operated on whilst avoiding all mechanical pressure in the region of the inclusion, to use also a suitable mounting medium

The stability of the body in the mounting medium seems to depend on the osmotic pressure Bodies were removed into solutions of NaCl, KCl or $CaCl_2$ of 0 01–0 1 M In a 0 1 M solution the body persists, at 0 07 M disintegration is gradual, below 0 05 M it is very rapid

The effect of the *p*H of the mounting medium on the inclusions was also tested In a 0 01 M solution, they broke down at all degrees of acidity from 7 to 2 2 In a 0 1 M solution the bodies remained intact over the same range of *p*H values The bodies thus appear to be almost unaffected over a wide range The only effect noticed was that in the more acid solutions the bodies withstand maltreatment more readily than in a neutral solution

The facts that the stability of the body depends on the osmotic pressure of the medium containing it and also that it often forms a vesicle on breaking down, at first sight appear to be arguments in favour of the existence of a membrane around the inclusion Its mode of formation, its appearance and extreme fragility do not suggest this

Striate material Tobacco mosaic disease produces in infected cells, in addition to the amoeboid *X*-bodies, flat plate-like crystals which were named striate material because cross-striations become visible under the action of acids (Iwanowski 1903, Goldstein 1926) These striations are present always, being visible in polarized light They are merely emphasized by reducing the pH value when the plate may actually break down into long needle-like fibres Beale (1937) has drawn attention to the morphological similarity between precipitated virus and the needles produced from the striate material

Similar plate-like crystals are produced by the breaking down of the large non-crystalline inclusions of aucuba mosaic disease Enation mosaic (Ainsworth 1937) produces in tomato X-bodies and striate material visually indistinguishable from those of tobacco mosaic The striate material of tobacco mosaic and of enation mosaic have been examined by micromanipulative methods

The application of slight mechanical pressure to the exterior of the cell in the vicinity of the plate results in its immediate breakdown into striae Pricking with a micro-needle causes a similar formation of needle-like fibres This is contrary to the results of Livingstone and Duggar (1934), who describe the formation of a granular mass on pricking

Attempts were made to isolate these plate-like crystals The medium used was a 0 1 M solution of potassium phthalate buffered to a pH of 3 4 In every case the plate broke down into long needles on being removed from the cell In a few cases the crystal was taken from the cell completely surrounded by cytoplasm Then it remained apparently intact although its striate structure was clearly visible (fig 5, Plate 28) Immediately an attempt was made to remove the cytoplasmic envelope the plate broke down into fibres It is improbable that these crystals could be successfully isolated even in a less acid mounting medium, as the drop in pH of the cell contents resultant on injury would probably cause the disintegration of the plate before it reached the medium

As the crystals could not be isolated it was impossible to test their virus content as was done in the case of the non-crystalline inclusions of aucuba mosaic disease

The virus content of the cell

Since intracellular inclusions were first observed much discussion has arisen as to their origin and nature They have been variously described as organisms causing the disease and as mere symptoms of the disease producing them The demonstration of the mode of formation of the inclusions of aucuba mosaic (Sheffield 1931) suggested that they are formed by the host cell as a response to the stimulation of the virus The question still remained as to whether the inclusions contain the virus, and if so, whether the virus is confined to the inclusions or whether it is also dispersed throughout the cytoplasm

It was intended to pick up small pieces from different parts of the cell contents and to compare the amounts of virus present This involved having a ready test for the presence of minute quantities of virus it was imagined that inoculation would provide such a test, but this soon proved totally inadequate It was then thought that the amounts injected were too small to contain the minimal dose necessary to cause infection But when quantities of virus certainly sufficient to produce the diseased condition, were injected by micro pipette directly into cells, in no case was more than one-tenth of the expected number of infections obtained (Sheffield 1936) The inoculation test was therefore temporarily abandoned, and a serological test similar to that designed by Matsumoto (1935) was tried This too proved unsatisfactory In order to carry out such a test with minute quantities of antigen it was necessary to watch the reaction under dark-ground illumination where it was found impossible to distinguish with certainty between an antiserum-antigen precipitate and the various granules and globules of the cytoplasm A ring test was then tried in the micro-pipette, a drop of antiserum being picked up after the antigen The end of the pipette containing the ring was broken off and mounted No ring was visible by transmitted light, and it was very difficult to make pipettes of the exact size to give a mount of the thickness necessary for dark-ground examination It was then decided to abandon the finer experiments planned, to collect larger quantities of extracted material, and to inoculate these into leaves which would show local lesions Purified virus was to be used as a standard with which to compare the extracted material This method was applied to *Solanum nodiflorum* infected with aucuba mosaic disease

The virus content of the inclusions was first examined It was essential first to determine the number of bodies necessary to give any appreciable number of lesions The figures of Bawden and Pirie (1937) indicated that, if the comparison were to be of any value, the dilution of virus should not be greater than 1 in 10^8 Assuming the inclusions to be spherical and of average diameter 20μ or (2×10^{-3}) cm, the volume of each would be $4(10^{-3})^3$ c c For such an approximation, the specific gravity can be assumed to be 1 The weight of each inclusion is then $4(10^{-9})$ g If each inclusion contains as much virus proportionately as the fibres, then to obtain a 1 in 10^8 suspension it is necessary to suspend ten inclusions in 4 c c of liquid

To obtain a number of inclusions of as uniform a size as possible they were taken always from the largest hairs and always from a cell in the same position in the hair For technical reasons the cell next to the basal one was chosen To remove a body a hair was selected in which the inclusion of the chosen cell lay near the base but preferably not actually on the cross-wall The whole hair was arranged in a hanging drop of 0 1 M potassium phthalate solution buffered to a pH of 3 4 The hair was held at the base by a large needle or by forceps so that it was flat against the cover-glass The hair was then divided into two pieces by a cut just below the top wall of the selected cell The upper part of the hair was pushed away and attention was confined to the portion held by the forceps This now consisted of a basal cell by which it was held and the cell from which the top wall was already cut and from which the inclusion was to be removed This was done either by exerting a very gentle pressure at the base of the cell or by replacing the cutting needle by a pipette and removing the body from the cell by suction The needles were then replaced by two pipettes both of sufficiently large diameter to admit the inclusion body and one of which contained phthalate buffer solution The inclusion was then picked up in the empty pipette and placed on a clean part of the cover-glass It was washed several times by flooding with liquid from the one pipette and removing the liquid with the second Finally, the inclusion was picked up in the pipette which had supplied the liquid for washing and was dropped into a tube containing a very small quantity of liquid Usually water was chosen, as in this medium the inclusions disintegrate and the substance composing them would presumably become evenly dispersed Until a sufficient number of inclusions was collected, the tube was stored in a refrigerator The suspension was then diluted to the desired extent, allowed to stand for some hours and then inoculated by rubbing on to leaves of *Nicotiana glutinosa*

For each of the first experiments ten inclusions were isolated, suspended in 4 c c of water and rubbed into the leaves A 1 in 10^8 solution of purified aucuba mosaic virus was used as a standard for comparison, and a water control was also used This experiment was repeated four times, and the results are summarized in the first column of figures in Table I

The second column of figures of this table shows the results of a similar but more detailed experiment, comparisons having been made at two different dilutions Twenty inclusions were isolated and suspended in 1 6 c c water 0 3 c c of this suspension were diluted to 1 5 c c to give a concentration the same as that used in the previous experiments These two suspensions were used as inoculum The standards for comparison were a 2×10^{-7} and a 10^{-8} dilution of aucuba mosaic fibres A water control was also employed making a total of five different treatments Inoculations were made on randomized half-leaves of *N glutinosa*, each treatment being replicated twelve times

TABLE I THE INFECTIVITY OF INCLUSION BODIES INDUCED BY AUCUBA MOSAIC VIRUS

Inoculum	No of lesions	
	60 leaves	12 half leaves
Water control	0	0
Virus fibres (1 in 10^8)	19	1
,, (1 in 2×10^7)	—	4
Inclusions (10 in 4 c c)	15	1
,, (10 in 0 8 c c)	—	7

The figures obtained from such experiments must inevitably be very small It was the work of several days to isolate the ten bodies used in each of the first series of experiments To obtain figures which could be subjected to statistical analysis, many hundreds of inclusions would need to be isolated The results obtained indicate quite definitely that virus is present in these inclusions

The lesions obtained were few in number, but none appeared in the water-inoculated plants The numbers of lesions caused by the suspension of inclusions were in each case of the same order of size as those produced by the standard dilution of fibres with which the inclusions were compared, suggesting that the inclusions contain a considerable proportion of virus

A comparison was then made between the virus content of the inclusions and that of the cytoplasm of the cell Hair cells of uniform size in which the inclusion lay against the cross-wall were selected Either that half of each cell which contained the inclusion or the half devoid of an inclusion was isolated When twenty of each had been collected, each set was suspended in 0 8 c c of water 0 15 c c of this suspension was later diluted to 0 7 c c As standards for comparison suspensions of aucuba mosaic fibres of concentration 1 in 10^7 and 1 in 5×10^7 were used and also a water control Inoculations were made on randomized half-leaves of *N glutinosa* Each of the seven treatments was replicated six times

In those cells used in the first experiment the inclusions were recently formed. No appreciable difference was found between the virus content of the two halves (Table II) A fortnight later the experiment was repeated, using cells 2 weeks older than those used in the first series The virus content of those parts of the cell devoid of inclusions seemed to have decreased in comparison with that of the virus-containing parts

TABLE II COMPARISON OF VIRUS CONTENT OF HALF CELLS CONTAINING INCLUSIONS WITH THAT OF THE REMAINING HALVES OF THE CELLS

Dilutions		Total nos of lesions per six half leaves			
Virus	Half cells	Water	Virus	Half cells devoid of inclusions	Half cells containing inclusions
		Younger cells			
1 in 5×10^7	3 in 0 6 c c	0	1	1	1
1 in 10^7	15 in 0 6 c c	0	4	3	5
		0	5	4	6
		Older cells			
1 in 5×10^7	3 in 0 6 c c	0	2	0	1
1 in 10^7	15 in 0 6 c c	0	8	2	6
		0	10	2	7

It would appear from these experiments that a relatively large quantity of virus is necessary to produce an infection The size of the virus particles is not accurately known, but the amount required obviously represents a large number of particles As all our present methods of inoculation are relatively crude no significance can yet be attached to this Woodruff and Goodpasture (1929), when testing the infectivity of isolated inclusions of fowl pox, obtained far fewer than the expected number of infections This failure was due to technical difficulties

Previous results (Sheffield 1931, 1934) had suggested that the non-crystalline inclusions of aucuba and tobacco mosaic viruses "consisted of material precipitated or coagulated from the cell sap or cytoplasm due either to the action of the virus itself or to some substance or a change in physical conditions produced by unbalanced metabolism resultant on virus infection" The body of aucuba mosaic was conceived as consisting of tiny particles of altered cytoplasm which for purely mechanical reasons became aggregated together The body was regarded as probably containing some virus, as, if the latter were dispersed throughout the cytoplasm, it would be reasonable to expect some of it to be swept into the body Results of these micrurgical studies necessitate a slight modification of this view The amorphous inclusions of aucuba mosaic contain relatively large amounts of virus But they are not homogeneous as chondriosomes can be demonstrated within them (Sheffield 1933) The stability of the inclusion over a wide range of pH values indicates that the virus they contain does not exist in the same state as that isolated in the test-tube (Bawden and Pirie 1937) which has an isoelectric point of 3 4 and is soluble at the pH of expressed sap The state of the virus within the inclusion and the possible nature of the striate material will be fully discussed elsewhere (Bawden and Sheffield 1939)

I am greatly indebted to Professor T Péterfi of Copenhagen for much invaluable advice in matters of technique when this study was first commenced

I must also thank Mr W G Cochran of the Rothamsted Statistical Department for assistance in designing some of the experiments and also my colleague, Mr F C Bawden, for kindly supplying the purified virus used

SUMMARY

Cells of virus-infected plants were examined by micromanipulative methods

The pH of the cell contents was found to be the same in diseased and in healthy plants

The non-crystalline intracellular inclusions of aucuba mosaic disease of tomato disintegrate immediately on slight mechanical pressure or on pricking They are almost unaffected by acids from pH 7 to 2 2 They break down if the osmotic pressure is reduced below 0 07 M but can be isolated into solutions of 0 1 M These inclusions contain virus but virus may also be dispersed through the cell

The striate material of tobacco and enation mosaics cannot be isolated, as immediately it is touched with a micro-needle it breaks down into needle-like fibres

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EXPLANATION OF PLATE 28

The photographs were taken with a Leitz Makam camera A Leitz 6L or a Leitz 2 mm objective was used in combination with a Leitz 10 × periplanat ocular, giving magnifications of × 450 or × 900 *Contact prints have been reproduced with a slight reduction*

The micro instruments were controlled by a Janse and Péterfi (Zeiss) micromanipulator

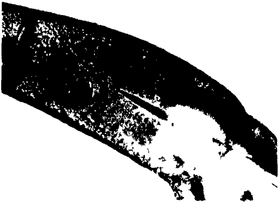
FIGS 1 *a–c* Cell from hair of *Solanum nodiflorum* infected with aucuba mosaic disease *a* A needle has been pushed through the transverse septum well into the cell Despite the injury to the cell the inclusion body was quite unaffected (× 383) *b* The inclusion was punctured by the needle which was immediately withdrawn again The body rapidly changed its consistency, forming a mass of clear bubbles in which a few granules were visible (× 383) (This photograph was taken about 15 sec after the body was pricked) *c* After 10 min the inclusion had almost disappeared, a few scattered granules only being visible (× 383)

FIG 2 Similar to fig 1*b* About 30 sec after pricking, the inclusion body had formed a single hyaline vesicle somewhat larger than itself Further disintegration, as in fig 1*c*, occurred later (× 383)

FIG 3 Similar to fig 2 About 30 sec after mechanical injury the inclusion had broken down into a clear hyaline material on the surface of which were a number of granules Later the appearance of the cell was as in fig 1*c* (× 383)

FIG 4 An inclusion body produced in *S nodiflorum* by aucuba mosaic virus was isolated from the cell This photograph was taken prior to washing the body (× 765)

FIG 5 Striate material produced by enation mosaic virus in tobacco was excised from the cell The crystal formed needle like fibres which were held together by an envelope of cytoplasm Directly this envelope was broken, the fibres separated (× 383)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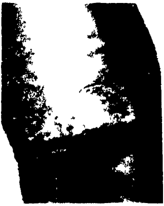
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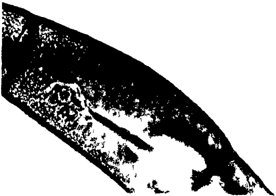
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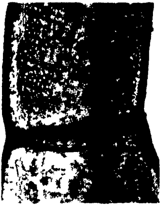
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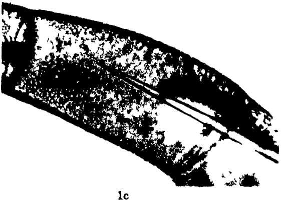
1c

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(*Facing p* 538)

611 013.2 593 95 *Psammechinus*
612 015.3

The metabolism of the eggs of *Psammechinus miliaris* during the fertilization reaction

BY H LASER AND LORD ROTHSCHILD
From the Molteno Institute and Zoological Laboratory, Cambridge

(*Communicated by J Gray, F R S—Received* 31 *August* 1938)

INTRODUCTION

Most of the work on the effect of fertilization on the metabolism of sea-urchin eggs has been concerned with the average rate of O_2 consumption before and after activation Although observations of this type are of interest, the changes which take place during the actual fertilization of the eggs are more indicative of the mechanisms responsible for the initiation of development Technical difficulties have impeded experiments of this latter type Early workers (Warburg 1915) found it necessary to fertilize the eggs before the beginning of the experiment, or, after measuring the O_2 consumption of unfertilized eggs, to open the manometer vessels to effect insemination The former method precludes any measurements during the fertilization reaction, and the latter involves a period of manipulation and consequent re-equilibration which exceeds the duration of the fertilization reaction When, therefore, it is stated that the O_2 consumption rate increases upon fertilization or parthenogenetic activation by some 200–400 %, what is usually implied is that the O_2 consumption rate measured over a considerable period of time after insemination is some 200–400 % more than the rate for a similar period of time before insemination

Shearer (1922) was the first to realize the importance of measurements immediately after insemination, 1 min after insemination he observed an increase in O_2 consumption which was not maintained at the original high level We have been unable to confirm Shearer's results when using a similar method (see fig 2) In recent years further attempts have been made (e g Runnström 1933*b*, Brook, Druckrey and Herken 1938) to make measurements immediately after fertilization by having the sperm inside the manometer vessel before fertilization but separate from the eggs, at a given moment sperm and eggs are mixed, but in these conditions, the shortest time interval measured after insemination was 10 min (Runnström), by which time the initial phase of the fertilization reaction is over.

There is a sudden evolution of CO_2 during the fertilization reaction which is mainly derived from the bicarbonate in the sea-water from which it is expelled by an acid diffusing out of the eggs (Ashbel 1929, Runnstrom 1933*a*) All experimenters so far* have measured the O_2 consumption by absorbing the CO_2 evolved with potash This method is satisfactory provided (1) that the CO_2 equilibration has been effected, (2) that the rate of production of CO_2 is slow compared with its rate of absorption by the potash, and (3) that its rate of production is fairly steady *Sudden* changes in the rate and amount of CO_2 production, as occur when these eggs are activated, tend to obscure the real O_2 consumption Although various authors have measured the O_2 consumption immediately after fertilization, only Gray (unpublished), apparently, observed this effect in practice Although the capacity of the potash would be ample for the absorption of the increased CO_2 output, the limitations on shaking rate imposed by delicate cells such as sea-urchin eggs make it impossible to re-establish a steady state of CO_2 absorption in the required time Therefore, the changes in respiration which occur at fertilization are unlikely to be detected either quantitatively or qualitatively by the use of this method

The possibility of measuring the O_2 consumption by non-manometric methods has been considered, but the quantities of O_2 evolved by suspensions of eggs which are sufficiently dilute to satisfy the desired biological criteria and the extremely short duration of the measurements make it doubtful if these methods would be superior to the manometric ones described in this paper

In order to find out what fraction of the total CO_2 evolved is derived from the respiration of the eggs and what fraction is derived from their acid production, it is necessary to measure the R Q and respiration at the same time Runnstrom has attempted to do this, using three vessels In the first, respiration was measured in the presence of potash, a procedure which has been discussed above In the second and third vessels, no potash was present and the total CO_2 was determined by tipping in a strong acid at times 0 and t The validity of this method depends on the assumption that respiration in the first vessel, in the absence of CO_2 and with a reduced bicarbonate activity in the sea water, is similar to respiration in the presence of CO_2 and with no alteration in the bicarbonate activity (Runnstrom's third vessel) The absence of bicarbonate appears to have a marked effect on the respiration of certain cells, respiration usually being higher when bicarbonate is present (Laser 1935, van Heyningen 1935)

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* With the exception of Brook, Druckrey and Herken, see p 541

In order to measure the total CO_2 produced, the so-called "retention" of CO_2 must be determined This retention is due to the presence of the eggs themselves, to small particles of jelly or shaken off fertilization membranes and to inorganic buffers in the sea water, causing some of the CO_2 produced to be chemically bound as bicarbonate (Warburg 1931) The value of this retention can only be determined when the liquid phase is in equilibrium with a given CO_2 tension in the gas phase If measurements are made of the retention of a medium containing bicarbonate such as sea water with *air* in the gas phase (Runnstrom 1933*b*), measurements include not only the retention but also to a certain extent the solubility of CO_2 in sea water

For these reasons it is preferable to measure the respiration of sea-urchin eggs in approximately normal sea water, i e containing bicarbonate, without absorbing CO_2, and when this sea water is in equilibrium with a gas phase of known composition containing CO_2 So far only Brook and others have attempted this, using Warburg's differential method, but with air in the gas phase Their paper appeared while this work was in progress They state that this technique is impossible to use because the eggs become damaged by the CO_2 which is present We have been unable to confirm this observation

In order to analyse the metabolism of the eggs during the fertilization reaction, it is necessary to measure the R Q at very short time intervals It is evidently useless to take an average post-fertilization value for the R Q when the metabolism changes its whole nature 5 min after fertilization A special technique has been evolved for this purpose When the O_2 consumption, total CO_2 production, R Q and retention have been evaluated, a picture of the metabolism of the eggs during the fertilization reaction may be obtained

MATERIAL

Ripe unfertilized eggs of *Psammechinus miliaris* were obtained by placing the ovaries of about five ripe females in a beaker of sea water, when the eggs diffuse out This suspension was passed once through bolting silk and was then washed 4–5 times in fresh sea water Eggs were fertilized in the experiments with mediumly dilute sperm suspensions No egg suspensions were used unless a sample showed 95% fertilization before the experiment, and the eggs in all experiments described in this paper showed 80–90% cleavage at the end, or after being left for the requisite time The eggs were counted by a method similar to that of Shapiro (1935), recently described by one of us (Rothschild 1939)

METHOD

General manometric procedure All experiments were done at 20° C The shaking rate was 85–90 cycles min $^{-1}$ The stroke was adjusted so that this rate did not damage the eggs After prolonged periods of shaking, normal development with synchronous formation of the 8- and 16-stages occurred Before taking the zero reading, the vessels were equilibrated for 12 min with the taps closed The metabolism of the unfertilized eggs was then measured for 15 min, sometimes at 5 min intervals, sometimes for one period of 15 min After 15 min the sperm suspension was tipped from a side-bulb into the egg suspension in the main vessel Three subsequent readings were then taken at 5 min intervals, and later readings at 10 and 15 min intervals

Experiments in the absence of CO_2 Ordinary conical Barcroft-Warburg vessels of about 13–15 c c capacity were used, they contained 3 0 c c egg suspension, 0 3 c c sperm suspension in the side bulb and 0 3 c c 10% KOH in the inner cup The latter also contained a roll of filter paper (Watmann no 40) protruding 0 5 cm over the edge of the cup and "flowered" to increase the absorbent surface The gas space contained air

Experiments in the presence of CO_2 A modification of Warburg's differential method (1925) was used

Normally different volumes of fluid in two vessels of the same size are brought into equilibrium with a gas containing a given CO_2 tension The O_2 consumption over a given period of time (XO_2) and the total CO_2 production (XCO_2) are calculated according to the formulae

$$XO_2 = \frac{h\ k\mathrm{CO}_2 - H\ K\mathrm{CO}_2}{\dfrac{k\mathrm{CO}_2}{k\mathrm{O}_2} - \dfrac{K\mathrm{CO}_2}{K\mathrm{O}_2}}, \tag{1}$$

$$XCO_2 = \frac{h\ k\mathrm{O}_2 - H\ K\mathrm{O}_2}{\dfrac{k\mathrm{O}_2}{k\mathrm{CO}_2} - \dfrac{K\mathrm{O}_2}{K\mathrm{CO}_2}}, \tag{2}$$

where h = readings of vessel with small volume of fluid,
$k\mathrm{CO}_2$ = CO_2 constant of vessel with small volume of fluid,
$k\mathrm{O}_2$ = O_2 constant of vessel with small volume of fluid,
H = readings of vessel with large volume of fluid,
$K\mathrm{CO}_2$ = CO_2 constant of vessel with large volume of fluid,
$K\mathrm{O}_2$ = O_2 constant of vessel with large volume of fluid

When working with cell suspensions it is preferable to fill the vessels with different amounts of the same suspension and not with the same amount of cell material in different amounts of fluid The addition of sperm in equivalent amounts to two vessels containing, for example, 5 0 c c (vessel I) and 1 0 c c (vessel II) of egg suspension led to difficulties, because if 0 3 c c of sperm suspension was used for vessel I, only 0 06 c c of sperm suspension could be used for vessel II, and this amount is difficult to mix with the main suspension Nor could the sperm for vessel II be diluted to 0 3 c c , (1) because the sperm suspension loses its activity too rapidly when diluted, and (2) because sperm suspensions at different dilutions respire at different rates (Gray 1928) Another difficulty was that the rate of shaking (85–90 cycles min $^{-1}$) necessary for equilibration in vessel I seemed in some cases to be too great for the cells in vessel II, where, owing to the small amount of fluid, the refraction from the walls was too vigorous and the cells were damaged The Warburg method was therefore modified in the following way instead of using two square vessels of equal size, containing different volumes of fluid, two round vessels of different size (12 and 25 c c) containing the same amount of fluid were used This modification has no theoretical objections and was entirely satisfactory

The introduction of a larger vessel with its associated larger constant produces an apparent loss of sensitivity when compared with the original Warburg method, but this is compensated by the omission of the factor (in this case 5) for the ratio of the fluids by which the readings referring to h have to be multiplied and which, when readings have to be taken at very short intervals, may partly counteract the advantage of the sensitivity In this method the readings h refer to the larger vessel, otherwise the same formulae apply

The gas space was filled with 0 3 % CO_2 in O_2 The effect of this mixture on the pH of the sea water was measured with a glass electrode and thermionic bridge (Rothschild 1937) and will be described later The gas mixture was passed for 15 min through the vessels containing eggs, etc while they were being shaken in the water bath The rate of the gas flow was about 200 c c min $^{-1}$ per vessel 12 min were then allowed for equilibration with closed taps before taking the zero reading After 15 more minutes, during which the respiration of the unfertilized eggs was recorded, the sperm suspension was tipped by two persons into both vessels at the same time, or, if the tipping were done one vessel after the other, the time interval between the tipping of the sperm in the two vessels was noted and subsequent readings taken with the same time interval

Measurement of CO_2 *retention* Warburg's method was used (1931) In this method, a known amount of CO_2 is liberated in the outer part of the vessel (1, fig 1) by mixing bicarbonate solution and acid The inner part (2) contains the egg suspension, etc which, according to its retention, reduces the pressure of CO_2 and thus gives a direct value for the retention The amount of acid which liberates CO_2 from the bicarbonate solution must be measured into the vessels with extreme accuracy The best way of doing this is to dry the acid in the side-bulb and then to weigh it, since the risk of error is considerable when pipetting *small* amounts In these experiments another but equally accurate method was used A large amount of weak acid (2 0 c c M/1000 citric acid) was pipetted into the outer part of the vessel, the side bulb containing 0 5 c c freshly prepared 0 025 M bicarbonate solution The inner part of the vessel contained 2 0 c c egg suspension + 0 12 c c sperm The vessels were filled with gas

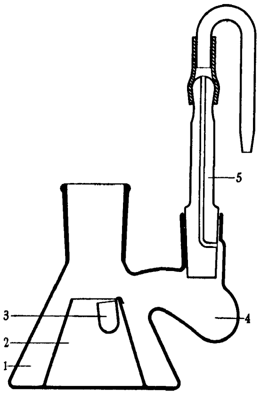

FIG 1 1, outer part, 2, inner part, 3, Keilin cup containing sperm suspension, 4, side bulb containing bicarbonate solution, 5, gas exit and equilibrated as described above A third manometer of the same size as the one in which the retention was determined, containing the same amount of eggs and sperm was run at the same time, in order to eliminate the pressure changes due to their metabolism during the experimental period

Measurements of R Q in combination with the differential method In order to determine the R Q in the presence of CO_2 for any given period, four manometers and the usual thermobarometers are required Vessels I and II are a pair of differentials as described above, the O_2 consumption is determined in them Vessels III and IV are conical vessels of about 18 0 c c capacity, having two side-bulbs each They contain in their main part 2 0 c c egg suspension and in the side-bulbs 0 3 c c sperm and 0 3 c c strong citric acid respectively All four vessels are filled with 0 3 % CO_2 in O_2 and equilibrated as described above At time 0 in vessel III both sperm and acid are tipped into the main vessel, in vessel IV the sperm only At time *t* in vessel IV the acid is tipped in The R Q can be derived from the following equation *

$$\frac{\left(\frac{\frac{a\ n}{m}}{KO_{2\,\mathrm{IV}}}+h_{\mathrm{IV}}\right)KCO_{2\,\mathrm{IV}}-(h\ \ KCO_{2\,\mathrm{III}})}{\frac{a\ n}{m}}, \qquad (3)$$

where m = amount of cell suspension in c c for which the O_2 uptake has been calculated in the pair of differentials,

a = O_2 uptake of m c c for the period in question,

n = amount of cell suspension in vessels III and IV respectively,

h_{III} and h_{IV} = the final manometer readings after tipping the acid into vessels III and IV

---

* This method can also be used to determine the R Q of glycolysing or non glycolysing tissue slices in Ringer bicarbonate in the presence of CO_2 Here, m = dry weight in mg of the tissue for which XO_2 has been determined in the pair of differential vessels, and n = dry weight of the tissues in vessels III and IV $a = XO_2/m$ mg dry weight of tissue Care must be taken that the weight (n) of the tissues in vessels III and IV is the same, which can easily be effected by adjusting their wet weight on a torsion balance before putting them into the vessels, or by choosing the same number of equal tissue slices for vessels III and IV

RESULTS

In Table I the *p*H of normal sea water, of sea water equilibrated with the gas mixture used in these experiments (0 3 % CO_2 in O_2), and of sea water shaken for 45 min in a closed manometer vessel in the presence of KOH are shown

TABLE I

Date		*p*H
30 vi 38	Sea water	7 96
	Sea water equilibrated with gas mixture	7 50
8 vii 38	Sea water	8 00
	Sea water equilibrated with gas mixture	7 50
9 vii 38	Sea water	7 99
	Sea water equilibrated with KOH	8 26

The gas mixture depresses the *p*H of the sea water, though the eggs develop quite normally up to the 16-cell stage in this medium The presence of KOH increases the *p*H of sea water, the equilibrium $HCO_3^- \rightleftharpoons CO_3^{=} + H^+$ being shifted to the right

As certain writers prefer to represent their results not per given number of eggs but per given amount of egg substance, measurements were made of egg diameters These measurements are shown in Table II As the unfertilized eggs are not absolutely spherical, measurements of maximum, minimum and mean diameters are given

TABLE II *P MILIARIS* EGGS μF DIAMETERS IN 10^{-4} CM (μ)

		Arithmetic mean	Standard error of arithmetic mean	No of observations
Female 1	Min diam	102 9	± 2 1	25
	Max diam	110 1	± 2 1	25
	Mean diam	105 4	± 1 8	50
Female 2	Min diam	101 4	± 0 9	25
	Max diam	104 9	± 0 9	25
	Mean diam	103 4	± 0 9	50
Female 3	Min diam	96 9	± 0 4	25
	Max diam	105 8	± 0 5	25
	Mean diam	101 6	± 0 4	50
Female 4	Min diam	101 1	± 0 9	25
	Max diam	104 6	± 1 2	25
	Mean diam	102 7	± 1 0	50

Thus 500,000 eggs are equivalent to about 0 29 c c of egg substance (see Korr 1937, p 465)

Fig 2 shows the typical O_2 consumption of an egg suspension in the presence of KOH At $t = 15$ min fertilization was effected, this being indicated by the arrow *F* The first reading on fertilized eggs was taken 2 min and the next 5 min after insemination For the first 2 min after fertilization, O_2 consumption apparently stops altogether In the next 3 min it continues at about the pre-fertilization rate, and it is only after this that the familiar increase in rate occurs

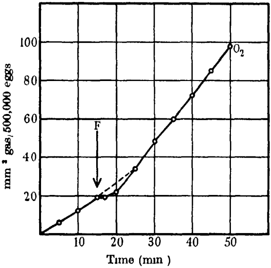

FIG 2 O_2 consumption in the presence of KOH *F*, fertilization Gas phase, air

This peculiar result, which appears immediately after fertilization, is entirely due to the sudden evolution of CO_2 caused by the reaction between the acid coming out of the eggs and the sea water * The CO_2 is not absorbed immediately by the KOH, and thus the O_2 consumption is masked If readings are taken only 10 min after insemination, this effect is barely visible, as is shown by the dotted line in fig 2 Furthermore, the immediate apparent cessation of O_2 consumption at the moment of insemination shows that the metabolic changes associated with fertilization occur immediately the eggs are fertilized, and that fertilization occurs in the manometer vessels immediately on insemination A further point may be noted about fig 2 The increase in rate of respiration after fertilization

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* We are satisfied that it is not the result of taking the vessel out of the water bath to mix the eggs and sperm, a procedure which takes about 10 sec

The respiration of the sperm suspension was negligibly small in all experiments is not so great as some workers have maintained This is due to the temperature at which the experiments were done (20° C) A few experiments were done at lower temperatures (15° C) and the usual increase was observed, but, as Korr (1937) noticed, when the temperature is increased, the disparity between pre- and post-fertilization rate decreases, and it is probable that if the experiment could be done at about 30° C there would be little difference in the rate before and after fertilization

The retention of CO_2 by the suspension of the eggs and sperm in sea water was measured in a number of experiments in the way previously described In about half the experiments the retention was 0 (see Table III) In a few it was found to be 0 02–0 03/1 0 c c , with an average retention of 0 01–0 02/1 0 c c A retention of such a low order can be neglected in equation (1) for the evaluation of XO_2 and XCO_2 No qualitative change is introduced in the results by this omission The introduction of the retention factor would slightly decrease the figures for XO_2, while the figures for XCO_2 would be slightly increased The changes would, however, be within the range of experimental error

TABLE III MEASUREMENT OF CO_2 RETENTION EXP 16, 24 VI 38

$T°$ C, 20 Gas, 0 3 % CO_2 in O_2 At $t = 0$, bicarbonate was tipped into the acid in vessels I and II, fertilization being effected by dislodging the Keilin cups containing the sperm into the inner part of vessels II and III

	I	II	III
Main vessel	2 0 c c M/1000 citric acid	—	—
Outer part	—	2 0 c c M/1000 citric acid	2 5 c c dist H_2O
Inner part	—	2 0 c c egg suspension	2 0 c c egg suspension
Side bulb	0 5 c c M/40 bicarbonate solution	0 5 c c M/40 bicarbonate solution	—
Volume of vessel, c c	13 760	24 785	25 000
KCO_2	1 266	2 255	—
h	+ 89 75	+ 50 75–0 5 = 50 25	+ 0 5

$$R\ (\text{per } 1\ 0\ \text{c c}) = \frac{(89\ 75 \times 1\ 266) - (50\ 25 \times 2\ 255)}{2\ 0 \times 50\ 25}$$

$$= \frac{113\ 4 - 113\ 2}{100\ 5}$$

$$\approx 0$$

In fig 3 the O_2 consumption and total CO_2 production are shown Inspection shows that there is either a respiratory quotient much greater than 1 0 before fertilization, or that there is a small amount of acid production, a point previously suggested by Ashbel (1929), though there is at

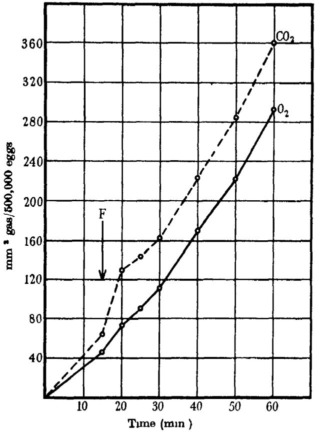

Fig 3 Total CO_2 production and O_2 consumption by differential method Gas, 0 3% CO_2 in O_2

present no evidence that this acid is glycolytic in origin, as this writer suggests At fertilization there is a sudden increase in total CO_2 production due to the production and diffusion of the acid out of the eggs This effect stops 5 min after insemination At the same time as the acid is produced, there is a marked increase in O_2 consumption This again is not maintained, and 5 min after fertilization the O_2 consumption rate falls to a low level which in some experiments was even lower than the pre-fertilization rate The O_2 consumption rate then increases to the normal post-fertilization level These changes in metabolism are not well seen in fig 3, where total CO_2 production and O_2 consumption are plotted "cumulatively", but in fig 4 the same experiment is shown with the amounts of O_2 consumed and total CO_2 produced at given time intervals plotted as rates per hour The two methods of expressing the data supplement each other, as although the first somewhat minimizes the significance of the changes in respiration, the second somewhat over-emphasizes them

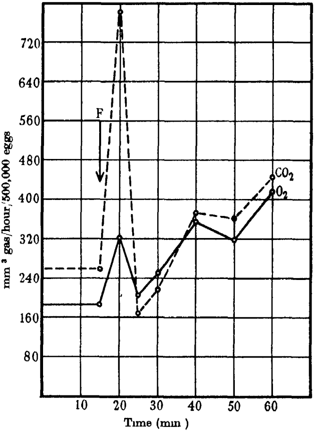

FIG 4 Total CO_2 production and O_2 consumption expressed as cu mm gas/hr, plotted against time Gas and method as in fig 3

In all experiments the initial outburst of CO_2 ceasing after 5 min, the initial increase in O_2 consumption rate followed by a sudden decrease and the subsequent increase were observed

Mention has already been made of the necessity for shaking at a fairly high rate, i e 90 cycles min $^{-1}$, in order to effect O_2 saturation in the vessel which contains the large amount of fluid This has been confirmed by experiments at a low speed of shaking (72 cycles min $^{-1}$), both with Warburg's original differential method described earlier, and with the new modification Experiments done in both ways show that the respiration was much lower than at a shaking speed of 90 cycles min $^{-1}$ (see fig 5), although the qualitative changes in rate after fertilization are the same When using Warburg's original method, the eggs in the small volume of fluid did not show a percentage of cleavage satisfying the criteria previously mentioned, and it is clear that the eggs became damaged some 10–15 min after fertilization, as the rates both of O_2 consumption and of CO_2 production decrease in a very anomalous way (fig 5 *b*)

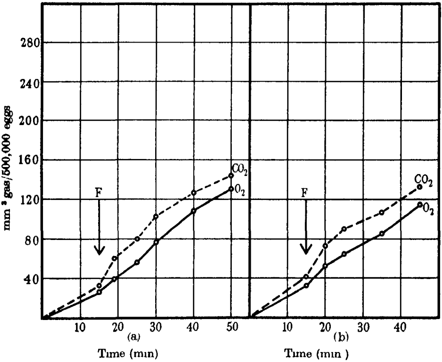

Fig 5 (*a*) Total CO_2 production and O_2 consumption at reduced shaking rate (72 cycles min $^{-1}$) Gas and method as in fig 3 (*b*) Total CO_2 production and O_2 consumption at reduced shaking rate (72 cycles min $^{-1}$) Gas as in fig 3 Original Warburg differential method

The R Q after fertilization has been determined in five experiments, each for different periods It was found to be well below 1 0 For the longest periods measured, 0–30 and 5–20 min after fertilization, the R Q was 0 84 and 0 66 respectively (see Table IV) For the periods immediately after insemination and during activation, i e for the first 5–10 min after tipping in the sperm, the values were much lower still The technical difficulties in measuring the R Q over such short periods of time (5 min) by means of four manometers and the usual thermobarometers is increased by the fact that an O_2 uptake of only a few cu mm has to be related to CO_2 pressures 20–40 times as great Since, however, none of the short period experiments which were satisfactory from a technical point of view showed a higher R Q , it is justifiable to state that the R Q immediately after fertilization is extremely low

TABLE IV MEASUREMENT OF R Q EXP 4, 10 VII 38

Vessels I and II, Warburg differentials Vessels III and IV, conical vessels each with two side bulbs $T°$ C, 20 Gas 0 3% CO_2 in O_2 At $t = 0$, sperm + acid were tipped into vessel III and sperm into vessel IV At $t = 30$, acid was tipped into vessel IV

	I	II	III	IV
Main vessel	5 0 c c egg suspension	5 0 c c egg suspension	2 0 c c egg suspension	2 0 c c egg suspension
Side bulb 1	0 4 c c sperm	0 4 c c sperm	0 4 c c sperm	0 4 c c sperm
Side bulb 2	—	—	0 3 c c 2 N citric acid	0 3 c c 2 N citric acid
Volume of vessel, c c	11 524	25 000	18 095	18 333
KO_2	0 588	1 843	—	1 572
KCO_2	0 990	2 246	1 750	1 773
$h(t = 30)$	− 32 5	+ 7 25	h + 57 0	+ 49 5

$$XO_2 = -104$$

$$\mathrm{R\,Q} = \frac{\left(\dfrac{\frac{104}{5} \times 2}{1\ 572} + 49\ 5\right)}{\frac{104}{5} \times 2}\ 1\ 773 - (57\ 0 \times 1\ 750)$$

$$= \frac{34\ 7}{41\ 55}$$

$$= 0\ 84$$

Two conditions must be realized if the results of these experiments are to be analysable (1) that eggs in each vessel fertilize at the same time, and (2) that eggs in both vessels fertilize at the same time For example, a small number of eggs being fertilized, say, 5 min later than the main bulk in both vessels, with concomitant acid production, would cause an apparent increase in the rate of CO_2 production and a false value for the R Q In order to check up that the above two conditions were realized, two differential experiments were done at the same time The results (fig 6) show that the experimental conditions are satisfactorily consistent, considering that the two sets of O_2 consumption and total CO_2 production curves are derived from four different vessels Though certain small disparities are evident, the changes in metabolism previously mentioned occur in both sets of curves in a very similar way Even the actual disparities which do occur look greater than they really are, because the number of eggs was approximately 350,000 per vessel, while the results are given per 500,000 eggs

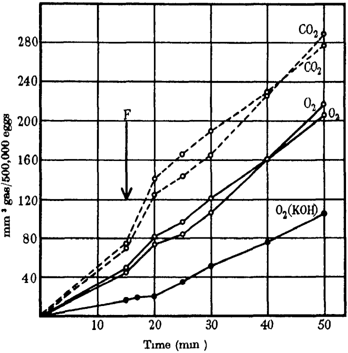

FIG 6 Three experiments running at the same time Total CO_2 production and O_2 consumption in duplicate and O_2 consumption in the presence of KOH

At the same time as the two differentials in fig 6 were running, an experiment was done in the presence of KOH There is a very marked decrease in respiration rate in the presence of KOH which reduces the HCO_3^- activity in the sea water and increases the *p*H A considerable number of comparisons of this type were done, and this disparity between the rates was always observed For this reason it seems probable that little attention can be paid to "absolute" rate of respiration when determined in the presence of KOH, as the eggs are clearly very susceptible to changes of this type in the medium Furthermore, the "absolute" respiration rates of egg suspensions from different batches of females was found to be variable, a fact also noted by Runnstrom (1933*b*), though it can be seen from fig 6 that the same egg suspensions show a very consistent respiration rate

DISCUSSION

The experiments described in this paper were done to gain further information about the first phase of the fertilization reaction, activation So far, very little except morphological details is known about it Whitaker (1933*a*) has shown that an increase in O_2 consumption rate after fertilization is not of general incidence The so-called increases in permeability to sparingly ionizing solutes (Jacobs 1933) are in the same category Biophysical changes, such as the increase of capacitance after fertilization (Cole and Spencer 1938), have only been recorded in one or two eggs There is some evidence that the respiratory mechanism changes after activation in the sea-urchin egg from a non-ferrous to a ferrous type (Korr 1937, Runnstrom 1930), and Örstrom (1937) showed that unfertilized sea-urchin eggs produce ammonia aerobically, while the fertilized eggs do not Apart from these somewhat isolated observations, little is known These experiments establish certain new facts related to activation So far, we do not know what their significance is with regard to the activation of the sea-urchin egg, nor can their general importance or significance be gauged until other eggs have been examined in the same way The sudden transient increase in O_2 consumption may be related to the sudden transient production of acid, and to the transient changes responsible for the extrusion of the fertilization membrane As both acid production and increased O_2 consumption last only for about 5 min, they might also occur in eggs such as those of *Cumingia* and *Chaetopterus*, which show a *decrease* in statistical O_2 consumption rate after activation Whitaker (1931, 1933*a*) was unable to measure the respiration of these eggs immediately after activation Apart from the acid production and increase in O_2 consumption, the apparent temporary change in R Q indicates that the metabolism of the egg not only changes after activation, but also that the metabolism changes again *after* the change due to the initial activation

With regard to acid production, the problem arises as to whether the acid is produced inside the egg at the moment of fertilization and diffuses out into the sea water, or whether the acid is being produced inside the egg before fertilization and some change in the cell membrane occurs at fertilization permitting the acid to diffuse out This latter hypothesis is reminiscent of the early concept that unfertilized eggs are in an "inhibited" condition which is rectified or changed by activation The fact that there is apparently a certain amount of acid production before fertilization might be held to support this view On the other hand, an increase in permeability to an ionizing solute such as a strong acid should be associated with a decrease in D C resistance, of which Cole (1928) has found no evidence, though a considerable time ago Gray (1916) found indications that some impedance change occurred The production of acid at fertilization is difficult to correlate with biophysical measurements, as Cole (1928, 1936) claims that the D C resistance of the cell membrane is exceedingly high, which does not fit in with the diffusion of a relatively strong acid On the other hand, Rothschild (1938) was unable to confirm Cole's results, though the method used was less refined A possible explanation of the discrepancy is that the permeability of the egg surface to ionizing solutes is a transient condition lasting only for a few minutes after activation, while Cole's most satisfactory measurements were performed on eggs that had previously been fertilized

One further point may be mentioned, the effect of the reduction in HCO_3^- activity (or increase in *p*H) has a marked effect on the O_2 consumption of sea-urchin eggs If a similar effect does not occur in other eggs, the relationship between the respiration of sea-urchin eggs and that of other eggs will have to be re examined Although the above-mentioned effect of changes in HCO_3^- activity occurs, the measurement of O_2 consumption during fertilization with a substituted buffer such as pyrophosphate or glycyl-glycine would be instructive, though acid production could not be measured manometrically In these conditions the technical difficulties imposed by the evolution of CO_2 derived from the bicarbonate in the sea water would be obviated, it is hoped to carry out experiments of this type in the near future

A possible criticism of these experiments might be that the change in *p*H caused by equilibrating sea water with a gas mixture containing 0 3% CO_2 in O_2 is just as great as the change caused by the presence of KOH Although this is true, the main effect of the CO_2 is to buffer the medium against possible changes due to the metabolism of the eggs There is no reason to expect the decrease in *p*H to increase respiration The main effect of the KOH is to reduce the HCO_3^- activity, and this might very well reduce respiration *

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* The disparity in O_2 consumption rates is not due to any deficiency in O_2 tension in air (the gas phase in KOH experiments) as compared with 99 7 % O_2, as Tang and Gerard have shown (1932)

We wish to record our thanks to Professor J Gray, F R S , for his advice and interest in this work, and to the Director and Staff of the Scottish Marine Biological Station, Millport, where the experiments were carried out

SUMMARY

1 The metabolism of *P miliaris* eggs was examined before, during and after fertilization

2 Immediately after activation, i e during the first 5 min , there occur

(*a*) a sudden increase in O_2 consumption,

(*b*) a sudden increase in acid production

3 Neither of these effects persists, the O_2 consumption falling to a low value, sometimes below the pre-fertilization rate This decrease in O_2 consumption is not maintained, the rate increasing about 10 min after activation to its normal value

4 There are indications that the R Q falls to an unusually low value immediately after activation

5 The comparative absence of HCO_3^- ions associated with manometric methods involving the use of KOH for the absorption of CO_2 in media containing HCO_3^- ions causes a large decrease in respiration rate

6 The relationship of these metabolic changes to physicochemical changes observed in the eggs at the same time is discussed

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Visual stimulation and ovulation in pigeons

By L Harrison Matthews

(*Communicated by F H A Marshall, F R S — Received* 27 *September* 1938)

It has long been known that the female pigeon, unlike the carefully selected domestic hen, does not ovulate spontaneously Harper (1904) showed that in pigeons ovulation occurs not less than 8 days after the introduction of the male to a female when both are ready for mating He further recorded that two female pigeons when confined together may both take to laying eggs The act of mating therefore is not necessary to induce ovulation, the mere presence of a companion sufficing This exteroceptive stimulus to ovulation is usually regarded as exerting its influence through the pituitary

The experiment described in this paper was designed to show whether the stimulus is visual, auditory, olfactory, tactile or a combination of two or more of these alternatives The essential part of the experiment carried the confining of two females together a stage further Two birds were confined in adjacent cages separated by a sheet of glass, and single birds were confined by themselves but provided with a mirror The birds were thus supplied with companions to which they had no tactile access

Wooden cages with fronts of half-inch mesh wire netting were used They measured 18 × 14 × 10 in Some of them were joined together in pairs, the partition between them being movable, so that it could be removed or a sheet of glass substituted for it Others were provided with slides at the back so that a sheet of mirror could be slipped in to cover the back wall

The birds used were dealers' ordinary stock, mongrels showing a predominance of the common blue rock They were placed in solitary confinement for a month, as a preliminary to the experiment, so that the effect of any previous stimuli might pass They were then confined as follows

Double cages
- (1) Male and female with no partition (control)
- (2) Male and female with glass partition
- (3) Two females with glass partition
- (4) Two females with no partition

Single cages
- (5) Female alone, with mirror
- (6) Male alone, with mirror
- (7) Female alone without mirror (control)

Owing to the difficulty of ascertaining the sex of mongrel pigeons a mistake was made with some of the birds One of those in cage no 3 turned out to be a male, so that this cage merely duplicated cage no 2 No 6 was confined as a female, but was found to be a male after the experiment had started Cages nos 1 and 2 were kept in a different building from the rest All the cages were arranged so that none of the birds could see any of those in other cages

The results obtained are shown in Table I, where the dates when eggs were laid are shown

It will be seen that eggs were obtained from the control pair in 9 days' time, and from other birds after longer intervals The female of the control pair was isolated on 12 June She laid again on 14 and 15 June, but thereafter produced no more eggs up to the end of July when the experiment ended The control female, shut up by herself with no mirror, did not lay at all up to 12 June On that day she was placed in a double cage with a male, and laid on 23 and 25 June The male (6), which was mistaken for a female and confined with a mirror, soon started calling and displaying in the usual manner of pigeons (cf Whitman 1919) to its mirror image

The results obtained show that the stimulus which causes ovulation in the pigeon is a visual one The ovulation of birds separated from the male by a sheet of glass, or confined alone with a mirror, shows that the stimulus is not tactile The absence of ovulation in the control, which was denied sight of another bird but was in the same room with the others, shows that the stimulus is not olfactory or auditory Were it either, or both, she would have laid Her subsequent ovulation when confined with a male showed her to have been a normal bird which lacked only the visual stimulus for ovulation

TABLE I DATES OF EGG-LAYING

Birds placed in solitary confinement 2 February Experiment started 3 March

1	2	3	4		5	7
control					♀ with	♀, no
♂ ♀	♂ glass ♀	♂ glass ♀	♀	♀	mirror	mirror
12 Mar	15 Mar	29 Apr	23 Apr		14 May	
13 Mar	17 Mar	1 May	25 Apr		16 May	
30 Mar	28 Mar	19 May		27 Apr	27 May	
1 Apr	30 Mar	23 May		29 Apr	28 May	
16 May	1 June	1 June	8 May		9 June	
18 May		4 June	10 May		11 June	
3 June		15 June		19 May	24 June	
		18 June		20 May		
♀ isolated		4 July	22 May			Placed with ♂
12 June			23 May			12 June
14 June				2 June		23 June
15 June				4 June		25 June
No further eggs to end of July			18 June			9 July
						10 July

Although visual stimulation from a companion of the same sex, or even from a mirror image, is sufficient to produce ovulation, it would appear that the response of the female to the behaviour of the male is also self stimulatory When the birds are denied access to each other, or are of the same sex, the pattern of the mating behaviour cannot be completed, and the lower level of stimulus produced is reflected in the longer interval before the appearance of the first eggs

It is claimed that this experiment lends further support to the explanation of sexual display in birds and other animals brought forward by Marshall (1936), who concludes that display does not produce a conscious sexual selection as proposed by Darwin, but is essentially a mutual pituitary stimulation The visual stimulation here shown to exist in pigeons when confined with a single companion, or merely a mirror image, is carried to a higher level in those birds which nest in colonies Fraser Darling (1938) showed that a definite minimum number of pairs is a requisite in any breeding colony of certain species of birds that nest gregariously, in order to produce the threshold stimulus for ovulation Visual stimulation has also been demonstrated in pigeons by Patel (1936), who showed that unless the male can see the female sitting on the eggs during incubation, he secretes no milk from the crop gland after the eggs are hatched

The writer wishes to express his thanks to Dr Richard Clarke, who kindly found accommodation for the experimental birds at the Clifton Zoological Gardens, and to Keeper Victor Jones who looked after them

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The photodynamic activity of the urine of mice treated with blastogenic hydrocarbons*

By J C Mottram

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(*Communicated by J W Cook, F R S —Received* 15 *October* 1938)

In view of the fact that a number of blastogenic agents and hydrocarbons have been found to make the skin of mice photosensitive and also to have photodynamic action on Protozoa (Mottram and Doniach 1937, 1938), examinations of the urine and faeces of mice either painted or inoculated with blastogenic hydrocarbons have been made for photodynamic substances The faeces may be dismissed by saying that no photodynamic substance was found, nor were the faeces seen to be fluorescent under Wood's glass

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* The term "blastogenic" is used to denote a tumour-producing agent, irrespective of the character or malignancy of the tumours produced All of the compounds so described in the present communication have been shown to give epitheliomas in mice, and with many of them sarcomas have been obtained by subcutaneous injection

As regards the urine, normal mice have been repeatedly examined for photodynamic action, with negative results A few cubic millimetres of urine can readily be obtained by manipulating the lower abdomen of mice, especially females, when this is diluted with a culture of *Paramoecium*, it is found that the organisms are killed in the dark in high concentrations, but that when a dilution of 16 parts of culture to 1 part of urine is used the organisms survive indefinitely and behave normally It was therefore decided to use dilutions of 1 part of urine to 32 parts of culture and 1 to 64 when looking for photodynamic action The urine of many normal mice, thus diluted with a culture of *Paramoecium* was exposed to the mercury-vapour lamp protected by a sheet of glass, and in all cases exposures for over an hour neither killed them nor interfered with their normal activity

A Pooled urine of mice painted twice a week with "impure" *not* light protected benzpyrene for 6 weeks, last painting 48 hr previously, these mice had been kept in the light (not direct sunlight)—the necessity for these details of exposure to light can only be fully appreciated by reference to previous papers (Mottram and Doniach 1937, 1938)

B Pooled urine of mice painted with impure benzpyrene similar to A, but of a different set of mice

C Pooled urine of mice painted as above but again of a different set of mice

D Pooled urine of normal mice for a control

	Dilution	Exposure	Result
A	1/32	M V L	Killed in 15 min
	1/64	M V L	,, 18 ,,
	1/32	Dark	Normal in 89 min
	1/64	Dark	,, 89 ,,
B	1/32	M V L	Killed in 14 min
	1/64	M V L	,, 24 ,,
	1/32	Dark	Normal in 82 ,,
	1/64	Dark	,, 82 ,,
C	1/32	M V L	Killed in 10 min
	1/64	M V L	,, 14 ,,
	1/32	Dark	Normal in 70 min
	1/64	Dark	,, 70 ,,
D	1/32	M V L	,, 67 ,,
	1/64	M V L	,, 67 ,,
	1/32	Dark	,, 76 ,,
	1/64	Dark	,, 76 ,,

M V L = exposed to the mercury vapour lamp Dark = kept in the dark

The conditions of exposure were lamp distance 21 cm , current 3 4 amp , 103 V , screen, glass opaque to radiations below 330$\mu\mu$.

When mice which have been painted with 3 4-benzpyrene are similarly tested, strongly positive results are obtained, as shown in the typical experiments on p 561

These results show the presence of a photodynamic substance in the urine 48 hrs after painting If these mice are examined 3 days after the last painting, then very little photodynamic substance is found, the 1/32 dilution for mice A killed in 62 min , for mice B the Paramoecia were slowed up but not killed in 69 min , and for mice C were normal in 53 min

When the mice were examined 4 hr after painting, then the times were A, 21 min , B, 15 min , and C, 10 min , showing that photodynamic substance is secreted in the urine soon after painting The rate of excretion is well shown by introducing one cubic centimetre of a 1/10,000 colloidal emulsion of impure benzpyrene into the stomach of the mouse, when the following results are obtained

Time when urine examined	15 min	1 hr	2 hr	3 hr	24 hr	48 hr	72 hr
Lethal time in minutes for 1/32 dilution of urine	Normal in 60	42	28	18	16	63	Normal in 60

So far only impure benzpyrene has been applied to the mice, since this itself contains water-soluble photodynamic substances produced by the action of light (Mottram and Doniach 1938), it cannot be concluded that the mice have changed the benzpyrene into a photodynamic product In order to settle this question mice painted with pure benzpyrene prepared and kept in the dark, and not containing water-soluble photodynamic impurities, were examined with the following results The mice had been painted twice a week for 5 weeks, pooled urine was used

Time after last painting when urine examined	4 hr	5 hr	24 hr	72 hr
Lethal time in minutes for 1/32 dilutions	27	16	18	Normal in 60

Pure benzpyrene was also tested, using mice which had been inoculated into both flanks with 0 5 c c of olive oil containing 1 mg of pure benzpyrene, pooled urine was used.

Time in days after inoculation when urine examined	1	2	3	4	6	8	10	13	27
Lethal time in minutes for 1/32 dilutions	4	8	13	10	8	26	13	4	9

These results indicate that after inoculation of pure benzpyrene in olive oil, the photodynamic substances continue to be excreted in the urine for at least a month, and that therefore they are produced in the tissues of the animals The wide variations in the figures are because individual mice vary though each has a certain constancy in pooling the urine the amount taken from each mouse was not kept constant and so this makes the readings of pooled urines variable

Next a series of polycyclic compounds, some blastogenic and some which do not produce tumours, were tested, by inoculating mice into each flank with 0 5 c c of olive oil containing 1 mg of the hydrocarbon and examining the urine 24 hr later, the results are given in the following table under urine A

	Urine A	Urine B	Blasto act	Photo act
(1) Cholanthrene	+	+	+ + +	+ + +
(2) 3 4 Benzpyrene	+	+	+ + +	+ + +
(3) Methylcholanthrene	+	+	+ + +	+ + +
(4) 2 Methyl 3 4 benzphenanthrene	+	+	+ +	+
(5) 1 2 5 6 Dibenzanthracene	Sl act	+	+ +	+ +
(6) 1 2 5 6 Dibenzacridine	–	+	+	–
(7) 3 4 Benzphenanthrene	–	Sl act	+	+ +
(8) 1 2 Benzanthracene	–	+	?	+ + +
(9) Anthracene	–	–	–	+
(10) Phenanthrene	–	Sl act	–	–
(11) 1 9 Dimethylphenanthrene	–	–	–	?
(12) Fluorene	–	–	–	–
(13) 1 2 Benzfluorene	–	Sl act	–	–
(14) Perylene	–	–	–	–
(15) Cholesterol	–	–	–	–
(16) Ergosterol	–	–	–	–
(17) Cholesterilene	–	–	–	–

Under Blasto act + + + = abundant tumour production, + + = definite tumour production, + = tumours occasionally produced, ? = tumours rarely produced, – = no tumours produced

Photo act = relative photodynamic activity of collodial preparations of the hydrocarbons, using *Coleps* for the test, see (2)

Urine A = deals with mice inoculated with 2 mg of hydrocarbons Sl act = slight photodynamic activity

Urine B = with 4 mg

It is seen that the first four hydrocarbons give urine having photodynamic activity, and that apart from the fifth, which shows slight activity, the remainder were inactive

A second series of mice were inoculated into each flank with 2 mg of the hydrocarbons in olive oil, and in the table under "Urine B" it is seen that now the fifth, sixth and eighth hydrocarbons give active urines, three showed slight activity and the rest were negative

In a previous paper (Mottram and Doniach 1938), the blastogenic activity of these hydrocarbons was compared with the photodynamic activity of colloidal preparations of them using *Coleps* for the test, and the data are again given in the last two columns of the table, as can be seen, between these two activities a correlation obtains which, as pointed out in the previous paper, is by no means perfect

In contrast to this, the correlation between blastogenic activity and photodynamic activity of the urine is very close, indicating that the blastogenic hydrocarbons are those, which the tissues are able to convert into a compound which, secreted in the urine, makes it photodynamic

DISCUSSION

The fate of inoculated hydrocarbons has been studied by a number of workers, Berenblum and Kendal (1936) using a fluorescence method could not find 1 2 5 6-dibenzanthracene in the excreta of mice after intraperitoneal inoculation, and Chalmers and Peacock (1936) likewise found nothing in the excreta of fowls after intramuscular inoculation of 3 4-benzpyrene and 1 2 5 6-dibenzanthracene Peacock (1936) detected a derivative of 3 4-benzpyrene in the liver and bile after intravenous inoculation, and in rabbits fluorescent substances, not normally present, were present in the urine when 3 4-benzpyrene, 1 2 5 6-dibenzanthracene and anthracene were inoculated intravenously Further, Chalmers and Peacock (1936) traced the fluorescent bile in chick embryos to the duodenum and in gradually decreasing intensity down the intestine Chalmers (1938) was able to detect fluorescent derivatives in the bile and faeces but not the urine of mice, 24 hr after intravenous inoculation of a colloid preparation of 3 4-benzpyrene

The present findings show that after subcutaneous inoculation of blastogenic hydrocarbons, photodynamic substances presumably derivatives of the hydrocarbons, continue to be present in the urine for at least a month

It is evident that elimination of these hydrocarbons requires further research, especially with regard to the chemistry of the derivatives present in the bile, urine, etc

Regarding the time required for the elimination of the hydrocarbons after inoculation, Chalmers (1934) observed a rapid decrease to one-tenth, after a few days when 1 2 5 6-dibenzanthracene, dissolved in chicken fat or egg yolk, was inoculated into the muscles of fowls Neither of these solvents are, however, good for the production of tumours, perhaps the elimination would have been much less rapid had for instance, olive oil been used

Chalmers and Peacock (1936) also observed rapid elimination in chick embryos after intramuscular inoculation in fatty solution of 3 4-benzpyrene and 1 2 5 6-dibenzanthracene Berenblum and Kendal inoculated mice intraperitoneally with 1 2 5 6-dibenzanthracene either in collodial solution or in lard They found that the hydrocarbon had mostly disappeared in 3 weeks, but in the case of the colloidal solution a quarter remained in the mice after 150 days

The present findings indicate that elimination is slow when inoculations are made in olive oil On the other hand, 3 days after the painting of skin, photodynamic substances are absent from the urine This rapid fall, after painting, is in keeping with the necessity of repeated paintings over long periods of time, for the production of tumours, and with the finding that painting twice a week is a good interval to adopt

The close correlation between blastogenic activity of the hydrocarbons and the presence of photodynamic substances in the urine, suggests that similar bodies should be looked for in the urine of cases of human cancer The present findings indicate that the blastogenic hydrocarbons are those which living tissues can convert to photodynamic derivatives, probably water-soluble oxidation products, since such are easily made, are soluble in water and are photodynamic, furthermore, Boyland and Levi (1937) have recovered a dihydroxy compound from the urine of rabbits fed on 1 2 5 6-dibenzanthracene

Now, Boyland (1933) has found that polycyclic hydrocarbons, oxidized photochemically, destroy dehydrogenases and that in this respect carcinogenic hydrocarbons are more active than the non-carcinogenic Again, Boyland and Boyland (1934) showed that a water-soluble oxidized product of 1 2 5 6-dibenzanthracene inhibited respiration (oxidation) and glycolysis of many different tissues Under discussion they say "As all known reactions involving enzymes take place in aqueous solution, it seems probable that carcinogenic hydrocarbons are altered in the body to form water-soluble compounds before they can produce biological effect " These observations on the urine of mice, decidedly favour this forecast

The expenses of this research were partly defrayed by the British Empire Cancer Campaign

CONCLUSIONS

1 Mice painted with pure light-protected 3 4-benzpyrene and kept in the dark, secrete a photodynamic substance in the urine for 2 days, but not for 3 days, after painting

2 Mice inoculated under the skin with pure light-protected 3 4-benzpyrene dissolved in olive oil, continue to secrete a photodynamic substance in the urine for at least a month

3 When a series of blastogenic hydrocarbons were thus inoculated, the urine was always found to contain photodynamic substances, but, when a series of hydrocarbons were used which do not produce tumours, then the urine remained normal in this respect The correlation was very close

4 Under discussion a number of facts are collected, which have a bearing on these findings and their interpretation

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END OF THE ONE HUNDRED AND TWENTY SIXTH VOLUME (SERIES B)

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

In accordance with a resolution of Council, summaries or abstracts of papers are to be published as soon as practicable The publication of such abstracts in no way indicates that the papers have been accepted for publication in any fuller form These abstracts will be issued for convenience with the "Proceedings of the Royal Society of London" but do not form a part of the "Proceedings".

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6 SEPTEMBER 1938

The effects of thunderstorms and lightning discharges on the earth's electric field By T W WORMELL (*Communicated by C T R Wilson, F R S —Received* 29 *June* 1938)

The results of an extended series of observations of the potential gradient during thunderstorms in the years 1926–36 are discussed A capillary electrometer was used to record photographically the behaviour of the gradient and continuous observations were possible even in the most intense fields and throughout heavy rainfall The effects of lightning discharges could be detected up to about 40 km distance The time of response of the recording system to a sudden change in the field was about 0 05 sec and the method thus yields an accurate record of the behaviour of the pre discharge gradient, of the recovery of the field after a discharge and also a certain amount of detail during the change of field accompanying a discharge

Records of a number of storms are reproduced and discussed in detail

The values of the pre discharge gradient beneath a thundercloud rarely exceeded 10,000 V/m even just before a close lightning flash Negative gradients were on the average more intense than positive ones at all distances from the storm When discharges were occurring within 5 km , negative pre discharge gradient was twice as frequent as positive, beyond 10 km , positive gradient predominated

The magnitude of the field changes due to very close discharges was very variable The biggest observed, of either sign, exceeded 50,000 V/m , but flashes within 1 km occasionally caused changes of only a few thousand volts per metre

The magnitude of the field changes at greater distances leads to an estimate of the electric moment of the discharges The mean value deduced for this quantity (i e twice the product of the charge by its vertical displacement) was about 220 coulombs × kilometres The values for positive and negative discharges were not greatly different

The most distant discharges recorded showed an excess of negative field changes (i e of changes tending to reduce the value of a positive potential gradient) For discharges within 15 km there was a considerable excess of positive field changes, the proportion increasing with decreasing distance until at about 5 km distance positive changes were more than three times as frequent as negative ones This well known behaviour is commonly interpreted as due to the frequent occurrence of a discharge involving the main positive charge at the top and negative charge in the base of the thundercloud For the very close discharges there was however, in the present series of storms, a marked increase in the proportion of negative field changes, positive and negative changes being about equally frequent for discharges within 3 km The observations would seem to demand the not infrequent occurrence of a second type of cloud discharge which causes a negative field change at short distances, but a positive change beyond its small reversal distance, and must be very low down

About one quarter of all the field changes recorded, even with this rather slow apparatus, showed components of both signs For very close discharges the proportion was considerably higher Such complex field changes were sometimes observed when only a single flash, either in the cloud or to earth, was visible in daylight Many of the simple field changes showed appreciable duration on the records, in a few cases exceeding 0 5 sec In some cases this was clearly due to the discharge being made up of several strokes but in others the change was recorded as a slow smooth variation of the field

The form of the recovery of the field after a discharge is discussed in detail and it is concluded that the initial rate of regeneration of electric moment by the cloud is, on the average, about 0 15 of the moment destroyed by the flash per second

A brief discussion is given of our present knowledge of some of the fundamental quantities connected with a thundercloud and of the theory of the mechanism of the production of the charges

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The use of a vertical pipe as an overflow for a large tank By A M Binnie (*Communicated by R V Southwell, F R S —Received* 30 *June* 1938)

The performance of vertical pipes arranged as overflows in a tank was studied experimentally on a small scale with special apparatus, which ensured that the water reached the pipe inlet with no tangential component of velocity

Under normal conditions the change of head with discharge was small at low heads At this stage the flow was not rotational, but a considerable volume of air was drawn down the pipe in the form of bubbles Above a sharply marked critical head, the pipe ran full and a large rise of head caused only a slight increase in the discharge The effects on the critical head of lengthening the pipe and of sharpening its inlet end were comparatively small, but the insertion of a trumpet shaped mouthpiece greatly improved the performance

The types of flow described by Borda as free and full flow were possible with overflow pipes of uniform diameter, and they resulted in a serious reduction in the discharge

The electromagnetic energy of a point charge By M H L PRYCE (*Communicated by P A M Dirac, F R S —Received* 30 *June* 1938)

A method of defining the energy of a point charge in a finite manner is developed It is roughly equivalent to subtracting an infinite constant from the ordinary expression in an unambiguous and relativistically invariant way The energy and momentum are quite simply given as the sum of a mechanical part and a (convergent) integral over all space For a point charge in uniform motion the energy and momentum are the same as for a particle possessing inertia

The equations of motion for a point charge are derived from the conservation of energy and momentum

In § 5 the theory is presented symmetrically with regard to the advanced and retarded fields

An absolute determination of the acceleration due to gravity at the National Physical Laboratory By J S CLARK (*Communicated by W L Bragg, F R S —Received* 5 *July* 1938)

An absolute determination of the value of g at the N P L has been carried out, using a reversible pendulum swinging *in vacuo* on a fixed knife edge, the plane bearing surface being part of the pendulum itself

The pendulum was designed to be as rigid as possible, and special attention was given to the calculation of the correction due to the elastic deformation of the pendulum when oscillating

Investigations were also made to determine the corrections due to curvature of the knife edges, elasticity of the support, amplitude of swing, and the residual pressure

The mean result of eighteen determinations gave the value of g at the N P L (lat 51° 25′ 14″ N , long 0° 20′ 21″ W , and height above M S L 10 m) as 981 181_5 cm /sec ^{2}

Specific heat-temperature curves of some age-hardening alloys By N SWINDELLS and C SYKES (*Communicated by W L Bragg, F R S — Received* 5 *July* 1938)

The changes in atomic configuration taking place during hardening affect the apparent specific heat of age hardening alloys Specific heat temperature curves and hardness measurements have been obtained on five typical age hardening alloys

The results show that in certain cases, e g the silver copper and copper beryllium alloys, maximum hardness is attained when the major portion of the chemical energy associated with the supersaturated solid solution has been evolved, i e precipitation has taken place In other cases, duralumin and aluminium copper alloys, maximum hardness is associated with segregation of the solute atoms in the parent lattice prior to precipitation

The classical equation of state of gaseous helium, neon and argon

By R A BUCKINGHAM (*Communicated by J E Lennard-Jones, F R S —Received* 11 *July* 1938)

The problem of deriving the form of the interaction of rare gas atoms from their observed second virial coefficients is attacked by the method introduced by Lennard Jones, using the classical equation of state and for the interaction energy

$$E(r) = \lambda r^{-s} - \mu r^{-t} \tag{1}$$

The method of calculation used by Lennard Jones for helium, neon and argon, assuming λ and μ constant, has been modified and then extended to allow for a possible slow variation of λ with r The bearing of the results upon the true interaction of helium atoms is discussed, mainly in relation to the accuracy of the Slater field For neon and argon it is emphasized that an unambiguous determination of the interaction energy requires an accurate knowledge of the properties of the crystalline form of these elements The description of the repulsive potential by analytic expressions, such as $be^{-r/\rho}$ and $b_1 r^7 e^{-r/\rho_1}$, is considered and parameters deduced which give the best correlation of gas and crystal data, on the assumption that (1) and kindred forms are valid for the interaction of neighbouring atoms only in the crystals

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The formation of negative ions by positive-ion impact on surfaces

By R PRESS and R H SLOANE (*Communicated by E V Appleton, F R S —Received* 12 *July* 1938)

It has been found that when a hot cathode discharge is passed through mercury vapour, light negative ions are produced from surfaces exposed to positive ion bombardment The negative ions include some with energies greater than could be imparted to them in the existing electric fields Experiments are described which eliminate the possibility that the excess energies could be produced through electrical oscillations The conditions under which the negative ions appear indicate that they are produced mainly through sputtering of occluded electronegative films under the action of mercury positive ion bombardment, but results from experiments of this type cannot be interpreted unambiguously The existence of this process has been shown directly by a double mass spectrograph in which beams of positive ions of known kind are allowed to impinge normally on a metal disk in high vacuum and are found to produce a spectrum of negative ions, with properties similar to those of the negative ions obtained in the previous experiments The existence of Hg^- as a stable entity seems to be still in doubt

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The porous diaphragm method of measuring diffusion velocity, and the velocity of diffusion of potassium chloride in water

By G S HARTLEY and D F RUNNICLES (*Communicated by F G Donnan, F R S —Received* 12 *July* 1938)

A continuous reading apparatus for measuring the rates of diffusion of pure electrolytes through porous diaphragms is described

Results obtained for the diffusion of potassium chloride as a function of concentration are presented and the absolute values obtained by extrapolation

The comparison of the results obtained with the best available data by absolute measurements shows that there is still room for more accurate work in the whole problem, but it is believed that the diffusion coefficients in the range from N/100 to N/10 are accurate to within 2 %

The determination of the size of paraffin-chain salt micelles from diffusion measurements By G S HARTLEY and D F RUNNICLES (*Communicated by F G Donnan, F R S —Received* 12 *July* 1938)

The porous diaphragm method has been applied to the measurement of the diffusion of paraffin chain micelles in aqueous solution with a swamping excess of simple electrolyte

For cetyl pyridinium salts the method is satisfactory and a radius for the micelle of about 26 A is found The radius is independent, within experimental error, of the concentration of paraffin chain or swamping electrolyte, and very little dependent on temperature These results are in excellent agreement with the spherical liquid micelle" theory There is small, but definite, influence of the nature of the gegenion on the size of the micelle

For cetane sulphonates and cetyl sulphates the method fails to give reliable results, probably owing to the building up of a structure within the pores of the diaphragm

When the solutions are saturated with amyl alcohol or benzene the method again fails to give reliable results A mechanism is suggested for the anomalies observed

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The simultaneous sorption of carbon bisulphide and water vapour by activated charcoals By A J ALLMAND, F R S and J L LIZIUS (*Received* 12 *July* 1938)

Although accurate work has recently been published in the *Proceedings* on the adsorption of *gaseous* mixtures on solid sorbents, the authors do not think that similar work of any value has appeared dealing with the simultaneous adsorption of two *vapours* They have attempted to carry out such measurements, using water and carbon bisulphide as the sorbates, and three activated charcoals of varying character as the sorbents The technique employed, including pressure measurements by means of a Pirani gauge, was an extension of that already described in the *Proceedings* of the Society

Experiments were carried out on the effect of varying charges of water vapour on the pressure exerted by a constant charge of carbon bisulphide sorbed on charcoal, on the sorption isothermals of water on charcoal containing such a charge of carbon bisulphide, and also on the sorption isothermals of carbon bisulphide on charcoal containing a constant charge of water The results are expressed in the form of diagrams They are of some complexity, due to the complications involved in the use of water vapour

The discussion is a somewhat detailed one, necessarily so in the opinion of the authors, in view of the complex nature of the systems studied It commences with a résumé of our present knowledge of the nature of the sorption of water and carbon bisulphide vapours by the charcoals of the particular types used—points to a parallelism between certain results in the present paper and the sorption isothermals of water on charcoal—makes detailed suggestions regarding the nature of the different stages of the latter (adsorption of single molecules passing gradually over to the formation of a continuous adsorbed film, with irreversible capillary condensation superposed)—explains the effect of an increasing water charge on the pressure of a constant carbon bisulphide charge in the light of these suggestions—shows how the isothermals of carbon bisulphide on charcoals containing sorbed water are consistent with the same views

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The mammary gland of the rhesus monkey under normal and experimental conditions

By S J Folley, A N Guthkelch and S Zuckerman

(*Communicated by W E Le Gros Clark, F R S —Received* 13 *July* 1938)

Examination of the mean mammary gland area of twenty six non pregnant normal female rhesus monkeys shows that over the body weight range 3400–6060 g the gland grows according to the simple allometric law The first menstrual cycles of the rhesus monkey after puberty are usually anovular Until ovulation occurs, mammary glands grow by proliferation of their ducts, and alveoli do not appear to form until after luteinization has occurred

Normally the mammary gland of the male rhesus monkey consists of a relatively uncomplicated duct system which does not extend far beyond the base of the nipple One otherwise normal male showed a condition of gynaecomastia, with clumps of fully formed alveoli Thirteen male monkeys were injected daily with varying quantities of oestrone for periods ranging from 6 to 484 days Only one of these animals (one that was injected for fewer than 15 days) failed to show duct proliferation Only one showed alveolar formation, no alveoli being present in the two animals which received the longest treatment (368 and 484 days respectively)

Only two of four female rhesus monkeys which were spayed before the beginning of the treatment with oestrone (37–365 days) showed alveolar formation Testosterone propionate appears to stimulate stunted duct development and alveolar formation in male monkeys

No carcinomatous changes were observed in the mammary glands of any of six monkeys which were injected daily with oestrone either continuously or for the major part of periods varying between 365 and 938 days

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The thermal decomposition of nitrous oxide

By C N Hinshelwood, F R S and R M Lewis

(*Received* 15 *July* 1938)

Further experimental data relating to the thermal decomposition of nitrous oxide at 747° and 652° are recorded

The variation of reaction rate with pressure is expressed both in terms of the reciprocal half time of reaction and in terms of the initial rates The two curves obtained are similar enough to show that previous conclusions based upon the half time curves are confirmed by the initial rate curve

The mean value of the activation energy falls at lower pressures, and the absolute magnitude of this energy agrees with most previous determinations, but not with those upon which has been based a theory that the reaction is abnormally slow for certain quantum mechanical reasons

The influence of the products of reaction and of additions of nitrogen, argon and carbon dioxide has been studied There is a contrast between the action of argon and that of carbon dioxide in that the former has a relatively greater effect at lower than at higher partial pressures of nitrous oxide This fact is consistent with a greater complexity of the reaction mechanism than corresponds to the assumption of a single "quasi unimolecular" reaction The nature of this complexity is discussed

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On the stability of flow between parallel planes

By D MEKSYN

(*Communicated by L Bairstow, F R S —Received* 20 *July* 1938)

The equations of motion of a viscous fluid for the case of flow between two parallel planes are considered, and it is shown that at sufficiently high Reynolds numbers and sufficiently small wave lengths, neutral vibrations of small amplitude can coexist with a laminar flow For moderately large values of R neutral vibrations can exist only for discrete values of R and λ, the wave length of the vibrations

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The band spectrum of silicon monosulphide and its relation to the band spectra of similar molecules

By R F BARROW and W JEVONS

(*Communicated by A Fowler, F R S —Received* 21 *July* 1938)

The spectrum of SiS is investigated in emission by heavy current discharges through a mixture of SiS vapour and argon In the region λ2585–3959 is a system of some 70–80 red degraded bands with single heads

$$\nu_{\text{head}} = 35028\ 8 + (512\ 0u' - 2\ 38u'^2 - 0\ 045u'^3) - (749\ 5u'' - 2\ 56u''^2)$$

(where $u = v + \frac{1}{2}$) That the lower state is the ground state of SiS is proved by the fact that some of the band heads (with $v'' = 0$ and 1) have been measured in absorption by SiS vapour In the region λ3491–6169 in the SiS discharge are about 70–80 red degraded bands, which form a part of a second extensive system, probably of SiS, with neither state in common with the first The vibrational frequencies for the upper and lower states are of the same orders for the two systems The first system almost certainly involves singlet states (either ${}^1\Sigma \rightleftarrows x^1\Sigma$ or ${}^1\Pi \rightleftarrows x^1\Sigma$), while the second system, which resembles the ${}^3\Sigma \rightleftarrows {}^3\Sigma$ system of S_2 in appearance, may involve triplet states of SiS The first system occurs also in heavy current discharges through Al_2S_3 vapour and argon, and through BCl_3 vapour and H_2S, in silica tubes, apparently without the second system The energies of dissociation of SiS in the ground state and excited state of the first system are about 6 0 ± 0 3 and 2 1 ± 0 5 eV respectively, and the products of dissociation are probably unexcited atoms, Si(^3P) + S(^3P) in each case The numerical constants for the ground states and certain excited states of the diatomic oxides, sulphides and selenides of group IV (B) atoms are surveyed, and from the empirical relations described the constants for the expected systems of SiSe and GeSe are estimated

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The behaviour of polar molecules in solid paraffin wax By R W Sillars

(Communicated by E V Appleton, F R S —Received 25 July 1938)

The experiments described constitute an extension of previous work by Jackson and were carried out on solid samples of paraffin wax containing small concentrations of various long-chain esters The esters are of molecular type similar to the wax, but have a permanent electric moment in virtue of the ester group Dipole effects similar to those found by Jackson are observed, and the variations of the characteristic time constants with temperature and with chain length of the esters are studied Variation with temperature is approximately exponential, the exponent being about the same for each ester It is found that the time constant is a non linear function of the total chain length of the ester molecule, but is relatively independent of the position of the ester group in the chain It is confirmed that the observed losses are due to dipole effects and not to some adventitious cause It is concluded that the whole ester molecule contributes to the time constant, and therefore that it behaves as a rigid structure and does not simply become deformed in the immediate neighbourhood of the dipole group This is in agreement with evidence from other sources The results do not suggest any simple relation between chain length and time constant, and a satisfactory theory to relate them has yet to be found

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The specific heat of β-brass By R Eisenschnitz *(Communicated by Sir William Bragg, F R S —Received 26 July 1938)*

A theoretical investigation is made of the specific heat of β brass The following assumptions are made in the course of the calculation The interaction energies of the atoms vary with the square of change of volume, the energy minimum of the average of Cu Cu and Zn Zn energies lying at higher volume than the minimum of Cu Zn

The partition function at constant volume is calculated according to the theory of Bragg and Williams, the corresponding partition function at constant pressure is obtained by taking an average over the variable volume

It is shown that the specific heat curve can be adjusted so as to give good agreement with experiment for temperatures below the maximum of specific heat

The theory of Bragg and Williams is applied to a lattice containing a finite number of atoms By introducing this number, agreement is obtained with the experimental slope of the C_p curve beyond the maximum A theoretical estimate is made which justifies the introduction of this finite number

A general theorem of statistical mechanics is derived, according to which all thermodynamical quantities in physically realizable systems are analytic functions of the temperature

The effect of solvents on the continuous absorption spectrum of bromine

By R G Aickin, N S Bayliss and A L G Rees (*Communicated by F G Donnan, F R S —Received 26 July 1938*)

The visible and ultra violet absorption spectrum of bromine has been investigated in the following solvents water (2N sulphuric acid), cyclohexane, chloroform, carbon tetrachloride, benzene, chlorobenzene, toluene

The weak ultra violet absorption of gaseous bromine is enormously enhanced in solution particularly by the aromatic solvents The smallest effect occurs in carbon tetrachloride, where considerable deviations from Beer's law were found Absorption maxima in the ultra violet were found at 2500 A in cyclohexane, at 2725 A in chloroform, and in the neighbourhood of 2950 A in the aromatic solvents

In the region of the visible continuum, the non aqueous solvents cause a marked increase in absorption intensity combined with a small or zero shift of the maximum towards the ultra violet Water causes a large displacement (1750 cm $^{-1}$) of the maximum to higher frequencies, but only a very small increase in intensity If the *A* component of the visible continuum is complex, the displacement of the maximum in non aqueous solvents can be interpreted as due to a greater solvent effect on $^1\Pi_u \leftarrow {}^1\Sigma_g^+$ than on $^3\Pi_{0_u^+} \leftarrow {}^1\Sigma_g^+$ The transition $^3\Pi_{1u} \leftarrow {}^1\Sigma_g^+$ is less affected still Difficulty is found in interpreting the observations if the *B* continuum is complex and *A* simple

Oscillatory motion of a fluid along a circular tube

By D G Christopherson, A Gemant, A H A Hogg and R V Southwell, F R S (*Received 26 July 1938*)

This paper is concerned with oscillatory motion (either free or forced) of a viscous fluid along a circular tube the restoring force comes in both instances from the "gravity head" which results from the passage of fluid Part I is a theoretical discussion of the problem Part II deals from a practical standpoint with complicating factors (e g turbulence, or meniscus and other end effects) which the theory does not take into account, and briefly discusses the possible application to viscometry Part III gives the results of experiments made to test the predictions of Parts I and II

The surface migration of barium

By M Benjamin and R O Jenkins (*Communicated by R H Fowler, F R S —Received 27 July 1938*)

It is found that under similar vacuum conditions barium does not migrate over tungsten, although thorium does It is also shown that barium does not migrate over nickel

The yield of mild steel, with particular reference to the effect of size of specimen

By J L M MORRISON (*Communicated by R V Southwell, F R S —Received* 28 *July* 1938)

The paper describes an investigation carried out to determine by means of tests under various conditions of uniform and non uniform stress distribution the criterion of yield in specimens of mild steel

Apparatus is described for heat treating material after machining in such a manner as to avoid surface decarburization, and for determining the stresses at yield of specimens tested in tension, compression, flexure, torsion and combined tension and torsion Experiments are described which indicate that the material used is uniform and isotropic, and the results of certain earlier investigations are compared with those found in the present paper

No differences are found between the yield of mild steel in tension and compression

The tests in combined tension and torsion give results in accordance with the theory of yield at a critical value of the shear strain energy stored per unit volume of the material It is later shown that this result is due to the size of specimen tested

Tests in tension on solid specimens of varying size and on thin tubes indicate that the character of the yielding of mild steel is a result of the polycrystalline nature of the material

It is shown that in all cases of non uniform stress distribution the yielding depends on the size of the specimen The explanation advanced to account for this result is that in such cases yield is delayed until a shear stress not less than the shear stress at yield under uniform stress is applied to a thickness of material of the order of a few crystal diameters

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The band spectrum of antimony fluoride (SbF)

By H G HOWELL and G D ROCHESTER (*Communicated by W E Curtis, F R S —Received* 28 *July* 1938)

The spectrum of SbF has been produced in active nitrogen and also by means of a high frequency discharge It consists of three groups of bands lying in the regions 3600–5000, 2600–2700 and 2200–2430 A Most of the bands in the first region had already been shown by Rochester to belong to two systems The remaining bands in this group are here allocated to a third system As the vibrational constants of these three systems are very nearly the same they are considered to be due to transitions between triplet electronic levels Similarly, the ultra violet bands form a triplet system, one component being in the region 2600–2700 A and the other two between 2200 and 2430 A The final state is common to both triplet systems and is probably $^3\Pi$ The multiplet separations of the states is not known nor is the nature of the other electronic levels

The vibrational constants of all the SbF systems are as follows

Visible triplet system

ν_e	w_e'	$x_e w_e'$	w_e''	$x_e w_e''$
21887 5	411 3	1 71	616 9	2 89
23992 5	420 0	1 75	612 6	2 58
27912 0	412 0	2 35	612 5	2 55

Ultra violet triplet system

ν_e	w_e'	$x_e w_e'$	w_e''	$x_e w_e''$
37937 6	696 9	1 09	616 6	3 19
43513 7	698 8	1 93	612 6	2 63
44750	700	—	612	—

The comparative behaviour of mammalian eggs *in vivo* and *in vitro* II The development of fertilized and artificially activated rabbit eggs

By G Pincus (*Communicated by J Hammond, F R S —Received* 3 *August* 1938)

This paper describes

(i) the sequence of changes occurring between sperm attachment and the first cleavage division in rabbit ova normally fertilized *in vivo*,

(ii) the cytological changes occurring in unfertilized rabbit ova during their sojourn in the fallopian tubes,

(iii) the cytological behaviour of unfertilized ova subjected to five activating treatments (exposure to (*a*) hypertonic solution, (*b*) supranormal temperatures, (*c*) treatment with rabbit sperm, (*d*) treatment with rat sperm, and (*e*) culturing in a moist chamber) is categorized, and the various events ensuing upon activation are described and discussed,

(iv) the effects of varying the intensity and duration of the hypertonic solution and heat treatments,

(v) the results of treating rabbit ova with the spermatozoa of six species of mammal other than the rat and rabbit,

(vi) a series of transplantations of artificially activated rabbit ova

These experiments are taken to demonstrate that (1) both ovarian and tubal ova activated by these treatments *in vitro* will in varying degrees exhibit development comparable to that of normally fertilized eggs, (2) the initiation of activation is an all or none process though its continuation probably is not, (3) haploid, diploid or tetraploid parthenogenesis results from the treatments employed, (4) the intervention of atretic processes may arrest the development of activated eggs at any developmental stage, (5) foreign sperm may activate rabbit eggs to a limited degree, but, except in certain instances with rat sperm, the foreign sperm do not penetrate the zona pellucida of the rabbit eggs, (6) living young may arise from artificially activated eggs, but only in a limited number of cases

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The heat of shortening and the dynamic constants of muscle

By A V Hill, Sec R S (*Received* 3 *August* 1938)

Further improvements in thermopile and galvanometer systems are described which have made it possible to read the initial heat production directly from photographic records, in absolute units and (for most purposes) without analysis

When a muscle shortens, in a tetanically maintained contraction, it liberates extra energy in two forms, (i) as "shortening heat", in amount proportional to the shorten ing, and (ii) as external mechanical work The shortening heat is independent of the load, and therefore of the work done and the speed of shortening The rate of energy liberation, i e of work + heat, is a linear function of the load, increasing as the load diminishes

The 'dynamic constants" of muscle, in a maintained contraction, are defined and measured They are

a, the shortening heat per cm of shortening,
P_0, the isometric tension,
b, the increase of energy rate per g wt of load

At any temperature these quantities are very constant a/P_0 is independent of temperature, b has a temperature coefficient of about 2 05 per 10° C A "characteristic equation" is deduced for the speed of shortening v under a load P, viz

$$(P+a)(v+b) = \text{const}$$

This equation is very accurately obeyed By fitting it to observations of v and P, the same constants a and b are obtained as are given by heat measurements

When a contracting muscle is made to lengthen gradually by applying a load rather greater than the isometric tension there appears to be a negative heat of lengthening, and the total energy given out by the muscle is less than in an isometric contraction The energy relations in lengthening are the converse of those in shortening and seem to be governed by the same rules

The nature of the shortening heat, and the mechanism by which the energy rate is governed by the load are discussed It is suggested that the chemical processes by which energy is liberated involve certain active points in the molecular machinery, and that when these are occupied in attracting one another and producing a tension they are not available to take part in chemical reaction

The work done by a muscle in raising a load, or in overcoming an elastic resistance, is transformed into heat in relaxation

The "visco elasticity" of contracting muscle is shown to be the property of a two-component system, the one component being undamped and elastic, the other being governed by the characteristic equation $(P+a)(v+b) = \text{const}$, and its corresponding energy relations The fact that an active muscle shortens more slowly under a greater force is due, not to "viscosity" but, as Fenn has claimed, to the manner in which the energy liberation is regulated A large force causes a low energy rate, which results in a low speed

From this can be deduced (i) the form of the isometric contraction, (ii) the form of the force distance curves made, at constant velocity, by the Levin Wyman apparatus, and (iii) that the greatest rate of doing external work should occur with a load equal to about 30 % of the isometric tension

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The specific heat of gases at high temperatures By R W FENNING and A C WHIFFIN (*Communicated by W L Bragg, F R S —Received 3 August 1938*)

(*a*) *Introductory* (*reasons for enquiry*) At the time this investigation was authorized there was some uncertainty as to the accuracy of the "accepted" values of the molecular heat, at high temperatures, of the gases which comprise the working fluid of the internal combustion engine, and in view of the experience that had already been gained at the N P L in connexion with gaseous explosions in closed vessels, it was recommended that a redetermination of this fundamental physical property should be attempted

(*b*) *Range of investigation* By carrying out explosions of mixtures of the type $(2+a)\,CO + O_2 + bA_r$ and replacing the argon in turn by CO, N_2 and CO_2, values have been obtained of the mean molecular heat of these gases between 373°K and about 2370, 2650, 2930 and 3060°K By balancing the heat losses in each group of explosions, the direct measurement of such heat losses was avoided although their magnitude could eventually be obtained from the heat capacity equations

Determinations have also been made of the mean molecular heats at constant volume of H_2 and H_2O (gas), the measurements being made from 373°K to temperatures ranging from about 2100 to 2900°K Although the highest temperature is considerably below that attained in the case of CO, N_2 and CO_2, it is believed to be a considerable advance on earlier work, the temperature extension being obtained by the substitution of nitrous oxide for oxygen in the explosive mixtures The H_2 and H_2O determinations are dependent on heat loss measurements for which a special device was employed

(*c*) *Conclusions* The values obtained for the mean molecular heats at constant volume of CO, N_2 CO_2, H_2 and H_2O (gas) are represented by the following equations over the specified temperature ranges

Between 373° K *and temperatures ranging from* 2350 to 3070° K

$$C_{CO} = 4\ 854 + 0\ 0_3 7725T - 0\ 0_7 875T^2,$$
$$C_{N_2} = 4\ 795 + 0\ 0_3 7675T - 0\ 0_7 875T^2,$$
$$C_{CO_2} = 8\ 631 + 0\ 001522T - 0\ 0_6 15T^2$$

Between 373° K *and temperatures ranging from* 2050 *to* 2930° K

$$C_{H_2} = 4\ 089 + 0\ 0_3 953T - 0\ 0_7 98T^2,$$
$$C_{H_2O} = 4\ 874 + 0\ 002284T - 0\ 0_6 256T^2,$$

where T = upper temperature in degrees Centigrade absolute

These values are compared with molecular heats deduced by other investigation from spectroscopic data and, for the ranges specified above, represented by

$$C_{CO} = 4\ 955 + 0\ 0_3 7018T - 0\ 0_7 812T^2,$$
$$C_{N_2} = 4\ 833 + 0\ 0_3 7224T - 0\ 0_7 822T^2,$$
$$C_{CO_2} = 8\ 672 + 0\ 001555T - 0\ 0_6 18T^2,$$
$$C_{H_2} = 4\ 369 + 0\ 0_3 6474T - 0\ 0_7 486T^2,$$
$$C_{H_2O} = 5\ 244 + 0\ 001893T - 0\ 0_6 194T^2$$

The difference between the two sets of values is comparatively small and if equal weight be given to each, the appropriate equations would be

$$C_{CO} = 4\ 904 + 0\ 0_3 7371T - 0\ 0_7 844T^2,$$
$$C_{N_2} = 4\ 814 + 0\ 0_3 745T - 0\ 0_7 849T^2,$$
$$C_{CO_2} = 8\ 651 + 0\ 001538T - 0\ 0_6 165T^2,$$
$$C_{H_2} = 4\ 229 + 0\ 0_3 8002T - 0\ 0_7 733T^2,$$
$$C_{H_2O} = 5\ 059 + 0\ 002089T - 0\ 0_6 225T^2$$

A further study of the problem of nuclear isomerism the application of the method of coincidence counting to the investigation of the γ-rays emitted by uranium Z and the radioactive silver Ag^{106}.

By J V Dunworth and N Feather (*Communicated by E V Appleton, F R S*—

Received 3 *August* 1938)

The application of the method of coincidence counting by electrical means to the study of the time correlation of nuclear processes—particularly that of successive quantum emission—is discussed in detail It is shown that a knowledge of the energy dependence of the sensitivity of the counters is essential for any interpretation of the results of such experiments For the γ ray counters employed in the present investigation some information on this point has been obtained by the use of sources of thorium active deposit of short and long exposure In the course of this work the general validity of the ideas of Ellis and Mott (1933) and of the nuclear level schemes of Oppenheimer (1936) has been established Also the paired quanta of the annihilation radiation of positrons have been utilized in the calibration

A study of the γ radiations from U Z and the long lived modification of Ag^{106} by the coincidence method has shown, in the former case, the emission of two quanta in succession in a very large fraction of the disintegrations, and, in the latter, the emission of four or five quanta in succession in the most probable mode This result is in general accord with the view that nuclear isomers are to be distinguished one from the other by a small difference in energy content and a large difference in angular momentum The particle emission from the short and long lived modifications of Ag^{106} has also been studied It appears probable that the negative electrons of the long period activity are internal conversion electrons associated with nuclear transitions following *K* electron capture, rather than that they represent a distinct mode of disintegration of the long lived radioelement, Ag^{106}

Relaxation methods applied to engineering IV Problems relating to elastic stability and vibrations

By K N E Bradfield, D G Christopherson and R V Southwell, F R S (*Received* 3 *August* 1938)

Preceding papers in this series have been concerned with unique solutions this paper deals with problems such that a complete solution would define an infinite number of configurations, all satisfying the governing equations and the terminal conditions, but each associated with some particular value of a critical loading or frequency (the "characteristic number", or *eigenwert*) The strut and vibrating rod (of non uniform section) have been chosen for detailed treatment as the simplest examples, respectively, of elastic stability and vibrations, but the principles as distinct from the details of our "relaxation" treatment are quite general, and they provide a new method of attack on problems which are at once difficult and important

In both problems the relaxation method appears capable of determining, with more than sufficient accuracy for practical purposes (when dimensions etc are not known exactly), not only characteristic numbers but also the mode associated with each (The modes are not determined by the well known theorem of Lord Rayleigh)

The nature of sliding and the analysis of friction

By F P Bowden and L Leben (*Communicated by G I Taylor, F R S —Received* 8 *August* 1938)

A detailed analysis of the kinetic friction between moving metals shows that the frictional force does not remain constant during sliding Sliding is not a continuous process the motion proceeds in jerks, and large and violent fluctuations occur in the friction In the case of dissimilar metals the top surface sticks to the bottom one and moves with it until, as a result of the gradually increasing pull, a sudden and very rapid slip occurs The process is then repeated indefinitely A simultaneous measurement of the surface temperature shows that this too is fluctuating and, at the instant of slip, there is a sudden temperature ' flash "

The exact behaviour depends upon the relative physical properties of the metals, particularly on the melting point, and there is evidence that three distinct types of sliding may occur The experiments suggest that friction is due to a welding together of the metals at the local points of contact These metallic junctions are large compared with the dimensions of a molecule and when they are broken the metal is distorted to a considerable depth

Even if the surfaces are lubricated with mineral oils or other lubricants an intermittent clutching and breaking away of the surfaces occurs and the behaviour may be essentially the same Certain long chain fatty acids may prevent stick slip and allow continuous sliding to take place

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The area of contact between stationary and between moving surfaces

By F P Bowden and D Tabor (*Communicated by G I Taylor, F R S — Received* 8 *August* 1938)

An estimate of the real area of contact between stationary and between moving metal surfaces has been made by electrical and by visual means Experiments with stationary surfaces show that the area of intimate contact is very small It varies with the pressure, but for flat steel surfaces it may be less than one ten thousandth of the apparent area The real area of contact is not greatly affected by the size, shape and degree of roughness of the surface it depends mainly on the pressure, The general behaviour is consistent with the view that the surfaces are held apart by small irregularities This means that even with lightly loaded surfaces the local pressure may be sufficiently great to cause the steel to flow plastically This conclusion is supported by the observed relation between the pressure and the electrical conductivity The slope of such a curve indicates that, at the points of contact, plastic flow of the metal has occurred Although the stresses may cause elastic deformation in the bulk of the metal, the experiments suggest that the irregularities on which the bodies are supported are crushed down until their cross section is sufficient to enable them to support the applied load

Measurements made with moving surfaces show that the area of contact is not constant but is fluctuating rapidly during sliding A detailed analysis shows a remarkable correlation between these fluctuations and the frictional and temperature changes described in an earlier paper It is clear that an intermittent clutching and breaking away of the surfaces is taking place and the results support the view that metallic junctions between the metals are being rapidly formed and broken The nature of these junctions depends on the relative physical properties of the two metals and there is again evidence that three main types may occur

The conductivity measurements show that even if the metals are lubricated with mineral oils and other lubricants, metallic contact may still occur through the film of lubricant When the surfaces slide, fluctuations in the area of contact are observed and the behaviour may be essentially the same as for unlubricated surfaces

Visual pathways concerned in gonadal stimulation in ferrets

By W E Le Gros Clark, F R S, T McKeown and S Zuckerman (*Received* 10 *August* 1938)

At the beginning of the non breeding season lesions were made at various levels in the visual pathways of the brains of female ferrets The animals were then exposed, at the end of each day, to bright artificial illumination for 6 hr and 30 min in order to discover whether or not they would come into heat during the anoestrum Normal control animals, and ferrets whose optic nerves had been divided were exposed to the same conditions, while normal control animals were also kept under ordinary laboratory conditions

The ferrets whose optic nerves had been divided either did not come into heat at all, or came into heat much later than the control animals All the other animals came into oestrus either within or soon after the control period set by the normal animals exposed to additional light The experiments showed that the normal response to visual stimulation occurs in the absence of the superior colliculi, when all retinal impulses to any part of the mid brain have been interrupted and when retinal impulses to the dorsal nucleus of the lateral geniculate body and the visual cortex have been completely interrupted Unless a few isolated normal cells in the dorsal nucleus of the lateral geniculate body provide the basis for adequate cortical stimulation, it was also found that a normal response can occur in the combined absence of the visual cortex and the superior colliculi, while another experiment strongly suggested that it can occur even after the interruption of retinal impulses passing to the dorsal nucleus of the lateral geniculate body, visual cortex, superior colliculi and pretectal area This being so, the conclusion is suggested that the visual response depends on impulses passing either to the ventral nucleus of the lateral geniculate body, or to the subthalamus by way of the accessory optic tracts

These results are discussed in relation to views on the exteroceptive stimulation of the pituitary in general and to the question of pituitary innervation in particular

ABSTRACTS

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23 SEPTEMBER 1938

Growth and development in the pig with special reference to carcass quality characters

By C P McMeekan (*Communicated by J Hammond, F R S —Received 8 August 1938*)

Carcass quality in the meat animal is defined, in terms providing a fundamental basis for its scientific study, as being dependent upon the proportions of the body and upon its relative composition in the terms of bone muscle, fat and offals On the hypothesis that these characters are determined by growth and developmental changes, and that such changes are themselves controlled by hereditary and environmental influences, a series of experiments was designed to provide the knowledge of the principles operative, essential to increasing man s effective control over the animal body

The composition of the bodies of some seventy closely inbred pigs of the Large White breed has been studied by complete anatomical dissection in the laboratory

Analysis of postnatal changes with age under normal conditions has established the general order in development of the body proportions, tissues and anatomical units The different parts and tissues exhibit marked differential growth, so that the existence of well defined growth gradients in the pig has been demonstrated

The influence of quantitative differences in the plane of nutrition, operative from birth for the first 16 weeks of life, is such that the differential response of the different parts and tissues is dependent upon their differential growth relationships

Complex experiments over the productive life of the pig as a meat producing animal afford convincing evidence of the influence of the nutritional environment as a directive and controlling force in the development of the animal body The shape of the growth curve is revealed as a dominant factor in this connexion Major modifications in form and anatomical composition do not occur as isolated effects but as orderly changes spread over a number of correlated parts

By establishing the quantitative relationships between "internal" and "external" body measurements, and the anatomical composition, a scientific basis has been given their use in the study of carcass quality in the pig

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A new theory of melting-points

By F C FRANK (*Communicated by N V Sidgwick, F R S*—*Received* 11 *August* 1938)

The only satisfactory existing theory of melting is the purely phenomenological thermodynamic description This reveals no connexion with existing molecular theories, such as Lindemann's, and stands to some extent in contradiction with these The modern recognition of order in liquids and disorder in solids raises the problem of explaining why there is an abrupt jump in physical properties on melting at all, instead of a continuous change, only showing discontinuity in a derivative quantity, e g in specific heat Peierls has already succeeded in showing that discontinuity in certain properties is necessary

In this paper the Bragg and Williams theory of order disorder transformation is applied to melting The basic assumption for this application is that the increasing disorderliness of a crystal near, and in fiction above, its melting point can be described by a continuous numerical measure An essential deviation from the cases considered by Bragg and Williams is that the lattice loses its rigidity on approaching states of low order This causes one to anticipate a change of form in the $V(S)$ curve of Bragg and Williams such that the transition is regularly one of first order, whereas their cases could readily be of either first or second order, in sensitive dependence on minor changes of circumstance

According to this interpretation there are critical temperatures above and below the melting point limiting the range of existence of the metastable (superheated and supercooled) states The probability that transformation will commence becomes great as the critical temperature is approached It is shown that the melting point may be expected to lie much closer to the upper critical point than the lower one, which may in certain circumstances be near the absolute zero This explains why it is impossible to keep a crystal in the superheated state, but sometimes necessary to supercool to very low temperatures to produce crystallization nuclei in a liquid

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Progressive lightning VI

By H COLLENS, D J MALAN and B F J SCHONLAND, F R S (*Received* 15 *August* 1938)

An account is given of some apparently anomalous lightning discharges observed with the Boys camera, and it is shown that their behaviour is in accordance with the same principles as govern the more usual type of discharge

Certain abnormal first leader processes occur in two stages, the first rapid and the second involving a velocity close to the limiting value for negative streamer propagation The first stage is considered to arise from the presence of space charge concentrations in the air below the cloud Dart streamers may travel down from the cloud during the second slow stage and catch up with the leader tip

Changes in the lower portions of the channels used by successive strokes of the same flash are shown to be associated with long time intervals between strokes, whereby the conductivity and guiding power of the previous track is destroyed A case of change in the upper portion of the channel is ascribed to the existence of a second centre of charge within the cloud

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The β-ray spectrum of Ra E By L H Martin and A A Townsend (*Communicated by T H Laby, F R S —Received* 15 *August* 1938)

The β ray spectrum of Ra E has been studied by means of a magnetic spectrometer, the magnetic field for which was provided by an annular electromagnet, and has a geometrical form especially suitable for β ray analysis

Special attention was paid to the effect on the distribution of back scattering in the source support, and absorption in the window of the counter The former was found to disturb the shape of the distribution to a greater extent than is generally appreciated With the source mounted on thin mica and after correcting for absorption in the window of the counter, the maximum of the distribution was placed at 1210 gauss cm , a value much further towards the low energy end of the distribution than has usually been found The average energy of the β particles is in good agreement, however, with the calorimetric values

Both the Fermi theory and the modification of Konopinski and Uhlenbeck completely fail to describe the experimental distribution The high energy limit was placed at 5395 gauss cm

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Optical thickness of the transition layer between transparent media By H D Bruce (*Communicated by J W McBain, F R S —Received* 16 *August* 1938)

In view of the use that is being made in recent years of the properties of reflected plane polarized light to determine the nature and thickness of the adsorbed or transition layer, the theories of Drude and Maclaurin are discussed and found to reduce to an identical form not permitting of mathematical separation of thickness and refractive index of the transition layer not even by the use of a series of observations as simultaneous equations It is shown that the minimum thickness of the adsorbed layer compatible with the equations is derived by assuming the refractive index of the adsorbed layer to be the geometrical mean of the indices of the two media

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The spectral variation of the photosensitivity of visual purple By C F Goodeve, R J Lythgoe and E E Schneider (*Communicated by C A L Evans, F R S —Received* 18 *August* 1938)

The photochemical bleaching of visual purple was measured quantitatively at wave lengths ranging from 440 to 560 mμ using the method of photometric curves described previously The experimental arrangement included a new high intensity Hilger monochromator

The results obtained for the photosensitivity of visual purple show that the quantum efficiency of bleaching is essentially constant and of the order of unity over the whole wave length range These facts lend further support to the previous arguments on the mechanism of the bleaching process

The spectral variation of the photosensitivity is compared with the scotopic luminosity curve and good agreement between them is found The significance of this result for the theory of scotopic vision is discussed

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A quantitative investigation into the enterohepatic circulation of bile salts in the cat By J MELLANBY, F R S, and S F SUFFOLK (*Received* 23 *August* 1938)

In a fasting cat about 95 % of the total bile salt is contained in the gall bladder bile The hepatic bile, continuously secreted by the liver, contains only traces of bile salt In the fed cat the volume and bile salt content of the hepatic bile shows a large increase, whilst the gall bladder contains only a small quantity of relatively dilute bile The removal of the small intestine causes an immediate fall in the cholic acid content of the hepatic bile to that found in the fasting cat

Bile salts are absorbed more rapidly from the ileum than from the duodenum and jejunum A small amount of absorption occurs in the large intestine, but the absorption of water takes place much more rapidly than that of bile salt Consequently the original concentration of bile salt may be increased fivefold in the unabsorbed bile Bile salts injected into the blood are rapidly excreted in the hepatic bile They are non threshold substances in respect of the biliary apparatus Bile salts are not stored in any tissue outside the enterohepatic system The intravenous injection of secretin increases by 50 % the volume of bile secreted by the liver It has no effect on the total quantity of cholic acid contained in the bile Its action is therefore that of a hydrogogue

Stimulation (acetyl choline) or paralysis (atropine) of the parasympathetic nerves to the liver has no influence on the volume or cholic acid content of the bile Stimulation of the sympathetic nerves to the liver (adrenalin) diminishes to a small degree the volume of the bile secreted but augments the total amount of cholic acid contained in it The sympathetic nerves may stimulate the formation of cholic acid from some inactive precursor by the liver cells

After removal of the bile from a fasting cat, two thirds of the cholic acid is re synthesized in 2 days if the animal feeds well In the absence of eating no synthesis of cholic acid takes place

A cat contains approximately 100 mg of cholic acid per kg body weight A rat contains approximately 25 mg cholic acid per kg body weight The rate of flow of bile per unit of body weight is about six times as great in the rat as in the cat

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10 October 1938

Determination of lung volume a simple constant volume modification of Christie's method By F J C Herrald and J McMichael (*Communicated by C G Douglas F R S —Received* 11 *August* 1938)

It has been shown that the method of estimating lung volume by oxygen dilution from a spirometer as recommended by Christie is liable to a considerable error owing to the fact that the volume of the whole lung spirometer system is undergoing a steady diminution and that even mixing of the gases is never achieved throughout the system, The error is most pronounced at functional residual air values over 3 l The error may be eliminated by adding oxygen to the system at a uniform rate so as to keep the volume of the lung spirometer system approximately constant throughout the mixing period

The standard error of single determinations is 0 186 l

The lung volume and its subdivision in healthy males By E A Aslett, P D'Arcy Hart and J McMichael (*Communicated by C G Douglas, F R S —Received* 11 *August* 1938)

The lung volume subdivisions of sixty four normal male subjects has been determined in the sitting position The results have been analysed statistically and correlated with various physical and radiological measurements From the regression equations, formulae have been estimated for predicting the more important lung volume subdivisions The best method of finding total lung volume is from a formula using the product of radiological chest area and antero posterior chest diameter, the vital capacity may be reasonably predicted from height or surface area

It has also been observed that the changes in body build occurring in obesity are associated with deviations from the more common arrangement of the lung volume subdivisions when these are expressed as percentages of the total lung volume

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Quantitative methods in psychology By LEWIS F RICHARDSON, F R S

(*Received* 20 *August* 1938)

In his opening address to a discussion on this subject (*Proc Roy Soc* B, **125**, 415, 1938) Dr C S Myers has asserted that ' We can never say that one experienced sensation of sound has twice the intensity of another "

A research on this question was made by L F Richardson and J S Ross (*J Gen Psychol* **3**, 288–306, 1930) The experimenter passed different electric currents of the same frequency through a telephone and the observer, who was kept in ignorance of the currents, was asked to assign numbers to the loudness in comparison with that of a standard current which by convention was taken to produce unit loudness Each of our eleven observers succeeded in making such numerical mental estimates One of the observers was Dr C S Myers himself and a summary of his results appears in the publication aforesaid The original laboratory notes show that I acted as experimenter towards him on 17 August 1928 The record of the mental estimates is in Dr Myer's handwriting, and shows that to different telephone currents he assigned loudness ratios ranging from $\frac{1}{8}$ to 6 One of his estimates happened to be 2 I had previously explained to him that Two would mean twice as loud"

Those who are in doubt about the possibility of quantitative estimates of mental events are requested to refer to the forthcoming interim report by a committee of the British Association (Sections A and J), where the question is argued from various some of them contrary, points of view I would particularly direct attention to Dr Semeonoff's summary of experimental work on intuitive estimates by independent investigators in England and America

This paper, being identical with its abstract, will not be published in the *Proceedings*

Micrurgical studies on virus infected plants By F M L SHEFFIELD

(*Communicated by R N Salaman, F R S —Received* 25 *August* 1938)

Cells of virus infected plants were examined by micromanipulative methods

The *p*H of the cell contents was found to be the same in diseased and in healthy plants

The non crystallic intracellular inclusions of aucuba mosaic disease of tomato disintengrate immediately on slight mechanical pressure or on pricking They are almost unaffected by acids from *p*H 7–2 2 They break down if the osmotic pressure is reduced below 0 07 M, but can be isolated into solutions of 0 1 M These inclusions contain virus, but virus may also be dispersed through the cell

The striate material of tobacco and enation mosaics cannot be isolated, for immediately it is touched with a micro needle it breaks down into needle like fibres

The development of the spark discharge II By T E Allibone and S R Meek (*Communicated by S R Milner, F R S —Received* 26 *August* 1938)

A further account is given of the development of the high voltage electric spark in air at pressures ranging from 76 to 0 3 cm Hg The account is based on photographs taken with a rotating film camera Many photographs were synchronized with voltage and current oscillograms of the spark

The leader stroke/main stroke mechanism established for the lightning flash and the spark at atmospheric pressure is shown to persist at all pressures down to 1 cm Hg The velocities of the leader strokes have been measured under a variety of circuit conditions The peculiarities of the oscillograms of breakdown first noted by Rogowski and his school are explained in terms of single and multiple leader stroke formation As the pressure is reduced the positive leader stroke develops at the expense of the negative leader stroke and the separate stages of the stepped leader stroke development are more clearly enhanced

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Ionic recombination in air By J Sayers (*Communicated by E V Appleton, F R S —Received* 26 *August* 1938)

An experimental study has been made of the recombination of ions in air It is found that, up to pressures of 1 atm , the recombination coefficient varies with pressure in a manner which is accurately predicted by the theory of J J Thomson, that is, the recombination coefficient varies proportionally with the pressure up to about 100 mm , thereafter varying less rapidly as the pressure is increased until, at 1000 mm , the recombination coefficient becomes pressure independent

The apparent variation of the recombination coefficient with the ionization intensity, noted by other workers, has been confirmed and explained as being due to the formation, by the ionizing rays of complex molecules such as ozone, new experimental evidence in favour of this explanation being adduced

A number of subsidiary experiments have yielded detailed information concerning the nature of the recombination process from which the inaccuracies in previous work on this subject may be appreciated

Using the new values of the recombination coefficient for air, it has been possible to calculate the equilibrium ion density and the electrical conductivity at various levels in the lower atmosphere For this purpose the rates of ion production measured by Regener by means of sounding balloons have been employed The results so obtained are shown to agree very closely with the direct measurements of the atmospheric conductivity made in the stratosphere ascent of " Explorer II " at South Dakota by Stevens and Anderson

Molecular interaction in monolayers III Complex formation in lipoid monolayers

By J H SCHULMAN and E STENHAGEN (*Communicated by E K Rideal, F R S —Received* 29 *August* 1938)

Two new methods are described for investigating the process of penetration of monolayers by substances injected into the substrate In one the monolayer is confined by a movable barrier and maintained at constant pressure by means of a non reactive piston fluid, the area change on injection being noted In the other an electrically operated Langmuir trough is employed, the slide retaining the monolayer travelling automatically so as to retain the monolayer at constant pressure during the process of penetration or ejection of one constituent

It is found that molecules containing ring systems such as digitonin, on penetrating a ring structure monolayer such as cholesterol form only 1 1 complexes, a number of cases of specific penetration in these systems are examined A saturated chain molecule such as sodium cetyl sulphate penetrating a ring structure monolayer such as cholesterol, forms a 1 1 complex The collapse pressure of the complex is found to be dependent on the concentration of the reactant in the substrate and to be in equilibrium with it The difference in surface free energies of formation of the complex with different bulk concentrations of reactant can be evaluated both from the surface pressure differences as well as from the variations in the bulk concentrations thus showing an experimental justification of Gibb s Law and that the complexes behave as chemical compounds Other examples of this type were found to form complexes of composition both of 1 2 and 1 1 Saturated chain reactants, e g sodium cetyl sulphate on penetrating saturated chain monolayers e g cetyl alcohol form stable complexes of composition 1 3 and 1 1, whilst an unstable 1 2 complex can be identified under certain conditions The rate of decomposition of a complex monolayer at constant pressure follows the unimolecular law

The presence of 1 3 and 1 1 complexes giving solid mixed monolayers with saturated chains and of 1 2 and 1 1 complexes with *trans* unsaturated chains suggests that the monolayer lattices of these complexes correspond to different ' crystal symmetries or types and if this proves to be the case on crystallographic examination Patterson's classification appears to be applicable

Marked differences, due to *cis* and *trans* isomerism, are obtained on penetration, supporting the concept of interlocking chains

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Haemocuprein and hepatocuprein, copper-protein compounds of blood and liver in mammals

By D KEILIN, F R S , and T MANN (*Received* 29 *August* 1938)

1 *Haemocuprein* is a blue copper protein compound present in the red blood corpuscles and serum of mammals

2 A method for isolation of this compound in a pure crystalline state from the red blood corpuscles of ox is described

3 A highly purified and partly crystalline haemocuprein may also be obtained from the red blood corpuscles of sheep and horse and the blood serum of horse

4 Pure haemocuprein contains 14 35% N, 1 12% S and 0 34% Cu

5 An almost colourless copper protein compound, *hepatocuprein*, containing 0 34% Cu, can be isolated from ox liver

6 Haemo and hepatocuprein have several properties in common Both have the same copper content, can be extracted from the crude material with the same alcohol chloroform mixture and purified by the same method In both compounds the copper is only loosely combined with the protein and is easily liberated by dilute trichloroacetic acid

7 They do not combine with molecular oxygen and do not exhibit any of the catalytic properties of enzymes such as peroxidase catalase, polyphenol and cytochrome oxidase or carbonic anhydrase

8 The possible biological significance of haemo and hepatocuprein is discussed in relation to the known effects of copper on blood formation growth and metabolism of organisms

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Critical and co-operative phenomena III A Theory of melting By J E Lennard-Jones, F R S, and A F Devonshire (*Received* 29 *August* 1938)

A mathematical theory of the change of phase from solid to liquid is given in terms of interatomic forces It is based on two former papers of this series (I and II), in which a statistical theory of a dense assembly whether solid, liquid or gas, was worked out Each atom was regarded as moving in a cell determined by its neighbours and the potential energy within this cell calculated as a function of the volume of the assembly

To this theory we have now added the concept of order and disorder and have discussed the relation between solid and liquid in terms of the amount of disorder present This has been achieved by regarding a solid as similar to an alloy in that it consists of a lattice of atoms and a lattice of "holes" the latter interpenetrating the first The energy necessary to interchange an atom and a hole is taken to be a function of volume This leads to a new contribution to the free energy and also to the pressure of the assembly due to disorder When this is added to the pressure calculated in former papers, the isotherms have a shape similar to those associated with a liquid vapour change of phase The theory gives the melting temperature as a function of pressure and shows that the change of phase is accompanied by an increase of volume and latent heat of fusion Calculations of these changes are made for argon and nitrogen and found to be in good agreement with experiment

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The metabolism of the eggs of *Psammechinus miliaris* during the fertilization reaction By H Laser and Lord Rothschild (*Communicated by J Gray, F R S —Received* 31 *August* 1938)

1 The metabolism of *Psammechinus miliaris* eggs was examined before, during and after fertilization

2 Immediately after activation, i e during the first 5 min , there occur

(*a*) a sudden increase in O_2 consumption

(*b*) a sudden increase in acid production

3 Neither of those effects persists, the O_2 consumption falling to a low value, sometimes below the pre fertilization rate This decrease in O_2 consumption is not maintained, the rate increasing about 10 min after activation to its normal value

4 There are indications that the R Q falls to an unusually low value immediately after activation

5 The comparative absence of HCO_3^- ions associated with manometric methods involving the use of KOH for the absorption of CO_2 in media containing HCO_3^- ions causes a large decrease in respiration rate

6 The relationship of these metabolic changes to physico chemical changes observed in the eggs at the same time is discussed

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On the Hamilton-Jacobi theory and quantization of a dynamical continuum

By P Weiss (*Communicated by R H Fowler, F R S —Received 1 September* 1938)

The Hamilton Jacobi theory of point mechanics is extended to the mechanics of continuous media, following on the lines first proposed by Prange The method is based on the equivalence between the Euler equations and the "boundary formula" (*formule aux limites*) in the calculus of variations It is shown that the notions of Lagrange brackets and Poisson brackets can be extended, but only if the Euler equations of the continuum are of hyperbolic type Consequently, these notions only apply to a dynamical continuum and not to equilibrium problems Except for this restriction, the method is applicable quite generally for linear as well as for non linear theories Once the dynamical laws are expressed in terms of Poisson brackets, the transition to the quantum theory can be effected by a brief formal argument in the same way as in point mechanics

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On the Hamilton-Jacobi theory and quantization of generalized electrodynamics

By P Weiss (*Communicated by R H Fowler, F R S — Received 1 September* 1938)

A procedure of quantizing a dynamical continuum, developed in a previous paper, is applied to the electromagnetic field in generalized form as given by Mie and by Born Important special cases are the Proca field of the heavy electron, whose structure is similar to the linear case of Mie's theory, and the Maxwell field which is the linear case of Born s theory In the theory of Mie, which is not gauge invariant, the stress energy momentum tensor suggested by the mathematical procedure differs from the one defined in physics, but it is shown that both tensors lead to the same physical results In the theory of Born, which is gauge invariant, the "potential" of the field is described by a line function $\oint \phi_i dx^i$ which has an absolute physical significance while the world vector (ϕ_i) has not In this way the ambiguity concerning the stress energy momentum tensor is avoided The well known peculiar difficulties due to the "antisymmetry" of electrodynamics can be overcome by a clear formulation of the correct initial data in the classical theory The present method is relativistically invariant throughout, so that an explicit proof of the invariance of the quantum conditions becomes unnecessary

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The electronic structure of some polyenes and aromatic substances VII Bonds of fractional order by the molecular orbital method

By C A COULSON (*Communicated by J E Lennard Jones, F R S*—*Received 3 September* 1938)

A new definition of the order of a bond in an aromatic compound is proposed, depending upon the energy change in the molecule when a slight variation is supposed to be made in the length of this bond This order is shown to be dependent upon the coefficients in the occupied molecular orbitals A curve is drawn relating the order and the length of a link and the method is applied to a discussion of the lengths and orders in the conjugated chain molecules $C_{2n}H_{2n+2}$

The emission band spectrum of chlorine III (continua)

By W H B CAMERON and E ELLIOTT (*Communicated by S R Milner, F R S*—*Received* 5 *September* 1938)

The spectrum of chlorine excited by active nitrogen has been photographed and measured The continua previously known were recorded together with a number of diffuse bands, hitherto unknown associated with the continuum whose maximum of intensity is at a wave length of 2580 A

An interpretation of these bands and associated continuum is given based on the assumption of transitions from a stable upper electronic state, probably analogous to the 'D ' state of $I_2(^1\Sigma_u^+)$ to a lower electronic state which has only a shallow minimum in its potential energy internuclear distance curve

A comparison is made between the chlorine spectrum excited by active nitrogen and by a high frequency electrical discharge

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The Australian aboriginal skull its non-metrical morphological characters

By FRANK J FENNER (*Communicated by F Wood-Jones, F R S*—*Received* 6 *September* 1938)

This paper consists of the records of 1182 adult Australian aboriginal skulls examined for their non metrical morphological characters, following the plan developed by F Wood-Jones

It was possible to distinguish three regional types of Australian skull the southern type, from South Australia, Victoria, New South Wales and probably southern Western Australia, the coastal Northern Territory type, and the Queensland type

The latter two varieties are thought to be differentiations from the original southern type by later extra continental racial admixture, with Melanesians in Queensland and possibly with Malays in Northern Territory Some support is lent to this suggestion by the published results of blood grouping of the Australian aborigines

The main bulk of the paper consists of detailed observations and cannot easily be summarized It is a contribution to the study of the racial differences of the human skull

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Investigations on magne-crystallic action VI Further studies on paramagnetic crystals

By K S KRISHNAN, A MOOKHERJI and A BOSE

(*Communicated by C V Raman F R S —Received* 5 *September* 1938)

The magnetic anisotropies of some typical salts of the rare earth and the iron groups have been measured at low temperatures down to about 90° K A simple cryostat, with automatic temperature control suitable for measurements on the magnetic anisotropies of crystals at low temperatures, is described The results are discussed on the basis of the theory of paramagnetism in crystals developed by Van Vleck, Penney and Schlapp

1 In those crystals in which the paramagnetic ions are all in the S state, e g $Gd_2(SO_4)_3\ 8H_2O$, $Mn(NH_4)_2(SO_4)_2\ 6H_2O$ the anisotropy $\Delta\chi$ is found to vary inversely as the square of the absolute temperature

2 In $Gd_2(SO_4)_3\ 8H_2O$ the maximum anisotropy, though only 1 4% of the mean susceptibility at room temperature, is still much larger than should be expected from the Stark separation of the S levels of Gd^{+++} under the crystalline electric fields The bulk of the observed anisotropy appears to arise from the magnetic interaction between the neighbouring Gd^{+++} ions

3 Chromic salts have a very feeble anisotropy, almost as feeble as if the Cr^{+++} ion were in the S state The criterion for judging the suitability of a paramagnetic salt as working material in the production of low temperatures by the Debye Giauque method is discussed

4 From measurements on the magnetic anisotropy of nickel salts at different temperatures, the strength of the coupling between the orbital and the spin angular momenta of Ni^{++} is calculated, and is found to agree with the spectroscopic value

5 Praseodymium and samarium ethylsulphates are of special interest As we go down to low temperatures their anisotropies increase at first, reach a maximum, and then diminish rapidly

6 The crystal fields in the rare earth chlorides, which are hexahydrated, are as strongly asymmetric as in the sulphates, which are octahydrated

7 Magnetic measurements on the cupric alkali chlorides enable us to decide between the alternative structures proposed for these crystals from X ray studies

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

In accordance with a resolution of Council, summaries or abstracts of papers are to be published as soon as practicable The publication of such abstracts in no way indicates that the papers have been accepted for publication in any fuller form These abstracts are issued for convenience with the "Proceedings of the Royal Society of London" but do not form a part of the "Proceedings"

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7 NOVEMBER 1938

The statistical mechanics of co-operative assemblies By E A GUGGENHEIM (*Communicated by R H Fowler, F R S —Received 5 September 1938*)

A regular assembly is defined as a co operative assembly with certain simple properties analogous to those of regular solutions The definition covers an important class of assemblies including, in addition to regular solutions mixed crystals, solutions of hydrogen in palladium and monolayers on a surface A general statistical treatment of regular assemblies is given the approximation involved being equivalent to that of Bethe The treatment given may be considered as generalizing, amending and, to some extent, simplifying the treatments by Bethe's method of various examples of regular assemblies due to Peierls, Lacher and Rushbrooke

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The β-ray spectrum of RaE By L H MARTIN and A A TOWNSEND (*Communicated by T H Laby, F R S —Received 15 August 1938*)

The β ray spectrum of RaE has been studied by means of a magnetic spectrometer, the magnetic field for which was provided by an annular electromagnet of a geometrical form especially suitable for β ray analysis

Special attention was paid to the effect on the distribution of back scattering in the source support, and absorption in the window of the counter The former was found to disturb the shape of the distribution to a greater extent than is generally appreciated With the source mounted on thin mica and after correcting for absorption in the window of the counter the maximum of the distribution was placed at 1210 gauss cm , a value much farther towards the low energy end of the distribution than has usually been found The average energy of the β particles is in good agreement, however, with the calorimetric values The high energy limit of the distribution was placed at 5395 gauss cm

Both the Fermi theory and the modification of Konopinski and Uhlenbeck completely fail to describe the experimental distribution

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Further experiments on olfactory conditioning in a parasitic insect the nature of the conditioning process By W H Thorpe, M A, Ph D

(*Communicated by A D Imms, F R S —Received* 14 *September* 1938)

In a previous paper it was shown that the ichneumonid parasite *Nemeritis canescens* can be induced to develop a positive oviposition response to the odour of an abnormal host, the Small Wax Moth (*Meliphora grisella*), by rearing it artificially upon that host and also by confining the newly emerged adult parasites with the Wax Moth larvae In the former case the conditioning effect was shown to be due in part to the influence of the host operating during the larval life of the parasite This being so it follows that the conditioning effect cannot be merely a simple reflex association with the act of oviposition, but is rather something in the nature of a "becoming aware" of a new factor (the odour of a new host larva) as part of a favourable environment The only comparable behaviour known in insects appears to be that described by Forel, Fielde and others in various species of ants The primary object of the experiments described in the present paper was to elucidate the nature of the conditioning process in *Nemeritis*

Adult *Nemeritis* reared from the normal host (*Ephestia*) were confined immediately on emergence in an apparatus through which was pumped a stream of air which had passed over a number of living *Meliphora* larvae In this way all possibility of direct association between the odour of the host and the act of oviposition was ruled out Nevertheless, when tested in the olfactometer these insects showed just as effective a conditioning as did those actually confined in contact with the host

The reaction of *Nemeritis* to the odour of various chemical substances was examined Cedar wood oil was found to be a convenient substance for experiment The odour of this oil is definitely repellant to the normal insect If, however, the *Nemeritis* are exposed to it in an air stream, a high degree of tolerance is produced without in any way injuring the animals

Within certain limits variation in the period of conditioning does not greatly alter the intensity of the final effect

The conditioning effect, both to *Meliphora* and to cedar wood oil, is highest immediately after the period of exposure On subsequent isolation it decreases gradually, with a considerable amount of fluctuation, approaching zero after a period of approximately 10 days

There is no definite evidence that younger insects are more readily conditioned than older ones It was shown that contact with *Ephestia*, after conditioning by this method to *Meliphora*, has an appreciable influence in reducing the intensity of the conditioning effect

By means of a modified olfactometer it was shown that the odour of *Nemeritis* exerts a slight attraction on its own kind It was also shown that the attraction that the webbing and faeces of the host exerts on the parasite does not apply so strongly to perception at a distance As far as odour alone is concerned the larvae of *Ephestia* are far more attractive than are the faeces and the web

It is concluded that conditioning in *Nemeritis* is not merely a simple association between the experience of a given constituent of the environment (e g odour) and the calling forth of a single specialised response (e g oviposition) It is rather the association of the given constituent with a favourable environment as a whole, not a specific element of it, thus giving rise to a positive response which will tend to keep the parasite in the type of environment in which the host itself occurs It seems that a similar concept provides the only satisfactory basis on which to explain the various well known observations on the behaviour of ants

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The adsorption of oxygen and hydrogen on platinum and the removal of these gases by positive-ion bombardment

By C W OATLEY (*Communicated by C D Ellis, F R S Received* 16 *September* 1938)

An account is given of the results of measurements of the contact potential difference between a tungsten filament and a platinum anode after the latter has been subjected to various treatments It is shown that a clean platinum surface can be obtained by successive bombardment of the anode with positive ions of oxygen and of argon The work function of a surface so prepared is found to be in good agreement with the most recent values obtained by thermionic measurements on clean platinum Values are also obtained for the work functions of oxygen on platinum, hydrogen on platinum and for mixed layers of oxygen and hydrogen on platinum The bearing of these results on the structure of the mixed layers is discussed

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The excitation of γ-radiation in processes of proton capture by light elements

By S C CURRAN, P I DEE and V PETRŽILKA (*Communicated by J D Cockcroft, F R S —Received* 19 *September* 1938)

Using a pair of Geiger Mueller counters in coincidence the excitation functions for the γ radiations emitted in the process of proton capture by each of the elements beryllium, boron, carbon and fluorine, have been determined for energies of the incident protons from 0 to 1×10^6 eV

Resonance peaks have been found in the excitation curves for incident protons of the energies given below

Beryllium		$350, 670 \times 10^3$ eV
Boron		$180, 650, 850, 950 \times 10^3$ eV
Carbon	^{12}C	480×10^3 eV
	^{13}C	570×10^3 eV
Fluorine		$330, 470, 590, 670, 860, 920 \times 10^3$ eV

Several of these resonance peaks had not hitherto been discovered In the cases of beryllium and boron a continuous excitation of γ radiation over the whole of the region of voltage employed was observed and evidence is presented in support of the view that this radiation must be associated with the capture process

The yield of γ radiation was measured in the case of carbon, the values obtained being $2\ 5 \times 10^{-10}$ quanta/proton for ^{12}C and $6\ 2 \times 10^{-9}$ quanta/proton for ^{13}C

A calibration curve is given relating the end points of the coincidence absorption curves (obtained by placing aluminium between the counters) with the maximum quantum energies of the incident γ radiations

A search for the production of radioactive ^{11}C as a result of the capture of protons by boron has shown that the cross section for this effect is at least 100 times smaller than the value which has been reported by other workers

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The combustion of methane By W E GARNER, F R S, and A J HAM (*Received* 26 *September* 1938)

The ignition limits for flame in mixtures of $CH_4 + 2O_2$ have been investigated over a range of temperatures and pressures The relationships between the pressure, temperature, and length of induction period in the region 640–750° are in agreement with the Semenoff equation, $p^n e^{-E/RT}\, T = \text{const}$ It is found, however, that whereas n is in substantial agreement with the value obtained by Neumann, E is much smaller

It is shown that the rate of change of pressure with time just before ignition and the maximum rate when ignition does not occur increases linearly with the pressure

Analyses have been made of carbon dioxide, carbon monoxide hydrogen, methane, oxygen, and formaldehyde present in the gases at various times during the pre flame, flame and after flame periods for the temperature 666° C and 8 cm pressure and from the results conclusions are drawn with respect to the course of the reactions and the generation of flame in the mixture $CH_4 + 2O_2$

A band spectrum attributed to NBr By A ELLIOTT (*Communicated by S R Milner, F R S —Received* 26 *September* 1938)

The spectrum of the after glow of nitrogen containing bromine vapour has been photographed

The vibrational structure of the band system so observed has been analysed, and the band heads fitted to a formula of the usual type

Considerations based on the isotope effect and on chemical evidence support the view that the bands are emitted by the molecule NBr

Visual stimulation and ovulation in pigeons By L H MATTHEWS (*Communicated by F H A Marshall, F R S —Received* 27 *September* 1938)

Pigeons do not ovulate spontaneously An experiment is described which shows that the sight of another bird, or of a mirror image, produces a visual stimulus presumably to the pituitary which leads to ovulation The bearing of this experiment on the subject of sexual display and courtship is pointed out

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A new theory of atmospheric temperature gradient By D S SUBRAHMANYAM (*Communicated by O W Richardson, F R S —Received* 5 *October* 1938)

A new type of vertical motion of air (which may be called "cumulative convection") different from that of "penetrative convection", is discussed The motion is assumed to be similar to that in long gravitational waves the volume of an element remaining constant during small displacements Vertical motion of this type is the result of difference of pressure from that corresponding to a state of equilibrium, set up in the vertical direction As a result of the change in pressure, the molecular energy of the element is altered which results in a change of temperature, and consequently in a lapse rate The value of this deduced for small displacements without acceleration is found to be $-\frac{dT}{dy}=\frac{g}{2JC_v}$, which gives on substitution a numerical value equal to 6 836° C/km This is in better agreement with the mean observed value, 6 71° C/km in the free atmosphere, than the one derived on the basis of penetrative convection, $-\frac{dT}{dy}=\frac{g}{JC_p}=9\ 86°$ C/km An explanation is also offered for the ascent of air masses in cyclones and their descent in anti cyclones

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Further investigations of very long waves reflected from the ionosphere By K G BUDDEN, J A RATCLIFFE and M V WILKES (*Communicated by E V Appleton, F R S —Received* 5 *October* 1938)

The paper describes an extension of the investigation of Best, Ratcliffe and Wilkes concerning the reflexion from the ionosphere of waves of frequency 16 kc /sec emitted from the British Post Office sender GBR at Rugby

An extension to greater distances of the previous measurements of the "Hollingworth interference pattern" formed at the ground, together with the removal of some instrumental difficulties in connexion with the "phase apparatus" mentioned in Paper I have led to more precise information about the downcoming wave The observations were explicable on the assumption that reflexion was from a height of

67 km for all angles of incidence between 30° and 60°, and was accompanied by a phase change of 180°, the reflexion coefficient of the wave component with electric field in the vertical plane was 0 12 on a September day, the wave was elliptically polarized so that the "electric vector ellipse" was described in a left handed sense, had its major axis in the vertical plane and the ratio of the axes was about 1 5 Propagation conditions were found to be similar in northerly and easterly directions

Observations on 127 days spread over the period July 1935 to July 1938 showed that the reflexion height varied during the day as though the waves were reflected from the lowest portions of a "Chapman region" at a level where the atmospheric "scale height" (H) was about 6 km (corresponding to a temperature of 180° K for air of normal composition) There was some evidence that the whole of the reflecting region was about 3 km lower in winter than in summer

Sudden anomalous decreases of reflexion height were sometimes observed and it was found that these showed a close relation to high frequency fade outs and other phenomena usually associated with catastrophic ionospheric disturbances

During and after severe magnetic storms or auroral activity the diurnal variation of reflexion height was found to be abnormal and the abnormality persisted for as long as 10 days During these abnormalities there was some evidence that the value of H was considerably reduced at the level of the reflexion

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Stress functions for a plate containing groups of circular holes By R C J Howland and R C Knight (*Communicated by G B Jeffery, F R S —Received* 6 *October* 1938)

A number of solutions of the biharmonic equation have been obtained, mostly in connexion with the problems of generalized plane stress, when the boundaries have consisted of circles and straight lines No general method of solution can be given but the methods which the present authors have used in certain cases are here extended to a group of problems The paper deals with circles in (a) the infinite plane, (b) a strip bounded by parallel lines The circles, their number and relative positions, are restricted by an invariancy condition, which demands that the circles and their boundary conditions remain invariant under one or more of a group of transformations and/or reflexions

In (a) the configurations have the boundaries (i) one pair of circles, (ii) two pairs of circles, (iii) a double infinite row of circles While in (b) (i) one pair, and (ii) two pairs of circles are dealt with These together with solutions previously published complete the group of problems to which the method is applicable

No numerical work is included but the expansions of the necessary functions have been determined They may be used for any problem where the biharmonic equation has to be solved with the appropriate boundaries The method of solution when the required functions have been established is indicated

Bakerian Lecture The structure of benzene By C K INGOLD, F R S

(*Received 7 October* 1938)

For many years attempts were made to explain the peculiarities of aromatic compounds by making various special hypotheses relating to the benzene nucleus It is a satisfactory feature of the present situation that the theory of benzene is simply a special (and specially important) case of an established general extension of valency theory, and that this extension uses essentially the same physical principles as are applied in the interpretation of valency itself These are the postulates of the 'new quantum theory (fundamentally the principle of complementality) which provide at once a physical basis for the forces of valency and for such departures from the ordinary valency rules as are illustrated so strikingly in the parent of the aromatic series

From an experimental point of view we are concerned to establish the correct stereochemical model for benzene, and to discover also in a quantitative way as much as possible about the internal forces which maintain that model Theory creates a predilection for a model of plane, regular hexagonal symmetry because theory works out most simply if such a model be first assumed The available experimental methods for the determination of a molecular model are (1) by X ray or electron interferometry, (2) from rotation vibration or vibration electronic spectra The interferometric method indicates the plane, regular model to a rather close degree of approximation, but the spectroscopic method, which is particularly sensitive and eminently suitable for application to this problem, was at first claimed to establish a contrary conclusion For this reason, and also because the spectroscopic method, by giving vibration frequencies supplies direct information about the internal forces, this method has been recently pursued by Angus, Bailey Wilson and others, and it has been employed in relation not only to ordinary benzene, but also to benzenes in which a part or the whole of the hydrogen is present in the form of deuterium This use of deuterium greatly increases the amount of information obtainable from the spectra, for isotopic substitution leaves the whole force system of the molecule unchanged, wherefore the vibrations which are responsible for the observed frequencies can be identified by comparing the frequency shifts caused by isotopic substitution with those calculated from the known changes of atomic mass under the assumption of unaltered forces (Teller) Four spectra are studied Raman scattering, infra red absorption, ultra violet fluorescence, and ultra violet resonance emission All give information concerning the molecular form and vibrations of the ground state of the molecule Five benzenes have been examined moderately completely, whilst two others (formulae in parentheses) are under examination C_6H_6, C_6H_5D, 1 4 $C_6H_4D_2$, 1 3 5 $C_6H_3D_3$, (1 2 4 5 $C_6H_2D_4$), (C_6HD_5), C_6D_6

By a comparison of frequency shifts and other spectroscopic characteristics amongst the spectra of these compounds, the frequencies of a large number of vibration forms have been identified From this information, with the aid of spectroscopic rules which depend ultimately on a consideration of the physical mechanism of production of the various spectra, it is possible unequivocally to establish the plane, regular model for the isolated (gaseous) benzene molecule The previously claimed contrary indication is explained as due to the use (or accidental presence) of liquid, wherein the intermolecular forces are sufficient to deform the molecules to a spectroscopically significant extent An approximate specification of the internal forces could be made at present, but in order to render such a specification complete with respect to harmonic forces, we require further identified frequencies, wherefore the extension involving $C_6H_2D_4$ and C_6HD_5 is necessary

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Dissociation, recombination and attachment processes in the upper atmosphere II The rate of recombination

By D R BATES, R A BUCKINGHAM, H S W MASSEY and J J UNWIN (*Communicated by E V Appleton, F R S —Received* 8 *October* 1938)

On the basis of the theory developed in a previous paper the effective recombination coefficient for electrons in the *E* and *F* regions is given by $\alpha_e + \lambda\alpha_i$ where α_e is the actual recombination coefficient for electrons, α_i that for ions and λ the negative ion electron ratio which, apart from small daily variations, remains constant In this paper the contribution to α_e arising from radiative recombination is evaluated by quantal methods, account being taken of the fact that an electron may recombine into any excited state of the positive ion It is found that this process is entirely inadequate to account for the observed recombination in both *E* and *F* regions Other possibilities are discussed and reasons are given for supposing that two body recombination of negative and positive ions, exemplified by

$$O^+ + O^- \rightarrow O' + O'',$$

is the most likely source of the observed recombination At the same time the ratio λ must be between 100 and 1000 for *E* region and between 10 and 50 for *F* Such values for *E* have also been suggested from terrestrial magnetic variations Lower values for *F* may be due to temperature ionization of negative ions or to inert constituents at *F* layer heights

The continuous absorption coefficient of atomic oxygen is also calculated

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25 NOVEMBER 1938

Summary of paper on two problems of extensional vibrations due to shocks

By W T HOWELL (*Communicated by Sir Robert Pickard, F R S* —*Received* 10 *October* 1938)

Heaviside's operational calculus is employed in the treatment of two problems of technical interest whose solutions do not appear to have been published hitherto, viz (1) a massive particle is attached to one end of a massive elastic rod, the other end of which is suddenly constrained to move with a constant speed, (2) a massive particle at rest is suddenly attached to one end of a moving massive elastic rod, the other end of which is constrained to move at a constant speed The conciseness of the method compared with that of the ' équation promotrice" of Saint Venant employed for allied problems is made manifest Formulae for the velocity of the particle and the extension of the rod at any time are given in each case and numerical examples illustrated with graphs are worked out for different values of the ratio of the mass of the rod to that of the particle In particular, it is shown in both cases that the particle may attain a maximum speed considerably greater than that of the other end of the rod

Summary of paper on diffusion in absorbing media

By P S H HENRY (*Communicated by Sir Robert Pickard, F R S* —*Received* 10 *October* 1938)

When one substance (e g water vapour) diffuses through another (e g air) in the pores of a solid body, and is at the same time absorbed by the solid, heat will be evolved This heat will itself diffuse through the body, and will affect the extent to which the solid can absorb the vapour There are thus two diffusion processes symmetrically coupled by the mutual interaction of the diffusants The definition of diffusion constants in absorbing media is discussed, and consequences of the coupling are worked out subject to certain simplifying assumptions The general solution is shown to be a linear combination of solutions to the ordinary diffusion equation with the same boundary and initial conditions but different diffusion con stants An analogy with the case of two coupled oscillatory systems is demonstrated A solution of the ordinary equation for diffusion into a rectangular parallelepiped is given which is suitable for numerical calculation, and is believed to be new A table of numerical data for diffusion into a plane sheet is given, from which values for a parallelepiped can quickly be deduced A numerical example of coupled diffusion is worked out for the diffusion of water vapour into cotton packed at different densities

The general theory of relaxation methods applied to linear systems

By G Temple (*Communicated by S Chapman, F R S —Received* 11 *October* 1938)

During the last few years Southwell and his fellow workers have developed a new method for the numerical solution of a very general type of problem in mathe matical physics and engineering The method was originally devised for the deter mination of stresses in frameworks but it has proved to be directly applicable to any problem which is reducible to the solution of a system of non homogeneous, linear, simultaneous algebraic equations in a finite number of unknown variables South well's "relaxation method" is one of successive approximation and, in order to complete the previous investigations of this method, it is necessary to prove that the successive approximations do actually converge towards the exact solutions this proof is given

Southwell's relaxation methods are not directly applicable to continuous systems, where the number of unknown variables is infinite, but it is shown here that simple extensions and modifications of the relaxation method render it suitable for applica tion to either discrete or continuous systems

The general theory of relaxation methods is then developed in terms of the theory of linear operators and sufficient conditions are prescribed for the convergence of the process of approximation

These general methods are then applied to the solution of non homogeneous linear in tegral equations and to the solution of non homogeneous, linear differential equations

Broadly speaking it appears that almost all linear systems of equations algebraic integral or differential can be brought within the scope of relaxation methods, which seem to constitute one of the most powerful methods of computation in mathe matical physics and engineering

The magnetic properties of bismuth III Further measurements on the de Haas van Alphen effect

By D Schoenberg (*Communicated by P Kapitza, F R S —Received* 11 *October* 1938)

The investigation of the field dependence of the susceptibility of bismuth (de Haas van Alphen effect) has been extended down to lower fields (1500 gauss) at helium and hydrogen temperatures, by measuring the couple on a bismuth crystal in uniform magnetic fields. The results are compared with Landau's theory, with which they are in good qualitative agreement.

Detailed examination shows, however, that there are discrepancies as regards (*a*) the phase of the periodic variation (*b*) as regards the details of the envelope curve of the periodic variation and the dependence of its amplitude on the crystal orientation, and (*c*) the temperature variation of the effect, which was not as rapid as predicted by the theory. From the comparison, we obtained the following estimates of the fundamental parameters of the electronic structure, for the "effective" electronic masses,

$$m_2 = 1.2\,m_0 \quad m_1/m_2 = 0.01 \quad m_3/m_2 = 0.02 \quad \text{and} \quad m_4/m_2 = -0.10$$

(m_0 is the ordinary electronic mass) and for the degeneracy temperature $E_0/k = 140°$ K. From these data the number of electrons responsible for the effect is deduced as 1.7×10^{-6} per atom. These results are compared with Blackman's estimates with which they are in agreement as regards order of magnitude, and their bearing on the previous experimental results is also discussed.

The photodynamic activity of the urine of mice treated with blastogenic hydrocarbons

By J. C. Mottram. (*Communicated by J. W. Cook, F.R.S.—Received 15 October* 1938.)

Several polycyclic hydrocarbons have been observed to photosensitize the skin of mice, and colloidal suspensions of them to photosensitize protozoa.

This action on protozoa provides a means of detecting them in very high dilution. Using paramoecium, the urine of mice painted with 3:4-benzpyrene has been examined and found to be photodynamic 1 and 2 days after painting, but not on the third day. On the other hand the urine of inoculated mice continues to be photodynamic for at least a month.

When mice are inoculated with a series of polycyclic hydrocarbons some of which are blastogenic and others inactive in this respect, the urine was found to be photodynamic only in the case of the blastogenic hydrocarbons. It appears therefore that mice are able to convert the blastogenic hydrocarbons into water soluble photodynamic derivatives which are excreted in the urine but are not able so to convert the non blastogenic hydrocarbons.

The spectrum of mono-deutero methane in the photographic infra-red

By W. H. J. Childs and H. A. Jahn. (*Communicated by Sir William Bragg, F.R.S.—Received 17 October* 1938.)

The preparation of 40 litres of mono deutero methane is described. With it the absorption spectrum in the photographic infra red has been investigated and measurements are given of a particularly simple band at 9021 cm^{-1}. From this band the moment of inertia of the molecule about an axis perpendicular to the

C—D axis can be deduced, and a value of 7 202 × 10^{-40} g cm^2 is obtained This corresponds (assuming a regular tetrahedral structure) to a C—H distance of 1 093 A, to be compared with 1 092 A in the case of CH_4 The band is assigned tentatively as the parallel component of $3\nu_3$, the second overtone of the perpendicular fundamental at 3031 cm^{-1}

Electron transfer in mercury vapour By F L ARNOT and MARJORIE B M'EWEN (*Communicated by H S Allen, F R S —Received* 17 *October* 1938)

An investigation of the electron transfer process

$$Hg^{2+} + Hg \rightarrow Hg^{+} + Hg^{+}$$

has been made using incident Hg^{2+} ions having energies from 140 to 400 eV The internal energy set free in the collision by the capture of the electron is 8 28 eV, of which it is found that about 6 3 eV are employed in exciting one of the resultant Hg^{+} ions, the balance of about 2 eV being shared as kinetic energy between the two Hg^{+} ions in accordance with the law of conservation of momentum

The mechanics of the collision process show that the results obtained can be accounted for only by a head on collision, one of the resulting Hg^{+} ions remaining stationary and the other carrying off the full kinetic energy of the incident Hg^{2+} ion plus the total amount of 2 eV internal energy available in the form of kinetic energy

The cross section for the process over the energy range of 140 400 eV has been obtained, and this shows an increase of cross section with energy of the incident Hg^{2+} ion, the value at the middle of the energy range being of the order of one tenth of the gas kinetic cross section for collision

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Refractive dispersion of organic compounds X Refractive indices of pulegone and of *m*-methyl*cyclo*hexanone Optical exaltation in unsaturated ketones By C B ALLSOPP and D M SIMPSON (*Communicated by R G W Norrish, F R S —Received* 17 *October* 1938)

Refractive indices over a wide range of visible and ultra violet wave lengths, including those covered by a ketonic absorption band, are recorded for pulegone and for *m* methyl*cyclo*hexanone The refractivities of the carbonyl radical in each compound are deduced and compared with those previously recorded for C=O in *cyclo*hexanone An enhanced refractivity in pulegone, which could be described as an "optical exaltation' in view of the conjugation of carbonyl and ethylenic double bonds, can be attributed to the presence of an absorption band which does not appear in the spectra of the other compounds, but equally abnormal values are obtained for *m* methyl*cyclo*hexanone for which this explanation cannot be valid It is concluded that these effects result from the invalidity of the additive relationship in the presence of the strongly chromophoric radicals, as predicted in a previous paper

Fused neurons and synaptic contacts in the giant nerve fibres of cephalopods.

By J Z YOUNG (*Communicated by E S Goodrich, F R S —Received* 20 *October* 1938)

Study of the structure of the system of giant nerve fibres in the squid shows that complete mingling of the neuroplasm of two nerve cells does occur, contrary to the strict tenet of the neuron theory Such fused neurons, however always function together in the animal impulses set up anywhere in them being conducted over the whole continuous neuroplasm This case therefore, is the exception to the neuron theory which proves the rule It shows that synapses, at which mingling of neuro plasms does *not* occur, are necessary as postulated by the theory, to prevent the indefinite spread of impulses

Very clear synapses are present in the giant fibre system and are described in detail The two first order giant nerve cells which set off the whole system are activated by numerous end feet scattered over their surfaces Presumably these neurons only discharge a propagated impulse when a sufficient number (and perhaps a certain pattern) of the end feet are activated In the stellate ganglion, however, a single impulse in the afferent member of the synapses produces a single impulse in the efferent and at these junctions there is provision for a very large surface of contact between the two fibres

The significance of the giant nerve fibres in the life of the cephalopods is con sidered, and the system is shown to one specialized for the production of movement rather than tonus Contractions are initiated by the action of the minimum possible number of motor units namely one pair of fused nerve cells A single impulse set up in this unit can produce a quick twitch of the retractors and expulsion of a jet of water from the mantle Such a system, though not well suited for the production of finely graded movements, allows the development of few, large and hence rapidly conducting fibres The combination of speed with fine gradation of movement is only possible in animals, such as vertebrates in which rapid conduction is obtained by thickness of the myelin sheath, rather than, as it is in the cephalopods, by great diameter of the fibres

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The absorption spectra of carbon dioxide and carbon oxysulphide in the vacuum ultra-violet

By W C PRICE and D M SIMPSON (*Communicated by R G W Norrish, F R S —Received* 21 *October* 1938)

The absorption spectra of CO_2 and COS have been investigated in the vacuum ultra violet In the case of CO_2, the electronic series converging to the lowest $^2\Pi_g$ state of CO_2^+ have been identified and the ionization potential corresponding to this state has been established directly as 13 73 ± 0 01 V This is in excellent agreement with Mulliken's prediction of 13 72 ± 0 03 V, which is based upon the identification of the 2900 A emission band as being $^2\Sigma_u^+ \rightarrow {}^2\Pi_g$ of CO_2^+, and upon the assumption that the photo ionization at 685 A is to the $^2\Sigma_u^+$ state Certain weaker bands not of a Rydberg type have also been found They are attributed to transitions to anti bonding molecular orbitals

The spectrum of COS has been found to be intermediate between that of CO_2 and CS_2 Certain differences are attributed to the lack of symmetry of this molecule The corresponding electronic states of the three molecules are identified by the criterion of the rough agreement of their molecular term values

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Collective electron ferromagnetism II Energy and specific heat

By EDMUND C STONER, F R S (*Received* 24 *October* 1938)

The treatment of collective electron ferromagnetism on the basis of Fermi Dirac statistics, applied in a previous paper to magnetic characteristics, is extended to energetic characteristics General expressions are developed for the electronic energy, which may be analysed into a quasi magnetic and a distributional part The forms taken by these expressions in the classical and Fermi Dirac limits are obtained, and general expressions are derived for the specific heat at low temperatures and at the Curie point

Detailed computations are made of the energy and specific heat over the whole temperature range corresponding to the critical value of the interaction for complete saturation at absolute zero Computations are also made, for a wide range of values of the effective interaction ratio of the energy increase between absolute zero and the Curie point, and of the specific heat at low temperatures, and immediately below and immediately above the Curie point

A brief survey of the specific heat results for nickel indicates that the theoretical treatment provides a satisfactory basis for the analysis and interpretation of experimental results for particular ferromagnetics

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Statistical theory of the adsorption of double molecules

By T S CHANG (*Communicated by R H Fowler, F R S —Received* 24 *October* 1938)

The adsorption of double molecules upon a solid surface with sites of accommodation regularly arranged is studied statistically allowing interactions between adsorbed molecules, by using Bethe's approximation Kinetic considerations leading to similar formulas are also given

The isotherms, when compared with those for adsorption of single atoms, seem to be bent towards the axis $\theta = 0$ When the forces between the adsorbed molecules are attractive, there is also a critical temperature T_c, below which the fraction of adsorption θ may change discontinuously when the pressure is increased The critical temperature is higher than that for the adsorption of single atoms, if the interaction energy between two atoms belonging to two different adsorbed molecules is the same as that between two adsorbed atoms for the latter case

It has been found possible by studying the particular case of no interaction, to get roughly the number of ways of arranging $\frac{1}{2}N\theta$ double molecules on N sites The result agrees fairly well with the exact value given by Fowler and Rushbrooke, who studied the number of arrangements at $\theta \simeq 0$, and $\theta \simeq 1$, and further it is found to be the same as the exact value for all θ, for the case in which the N sites are on a line It is applied when we use Bragg and Williams' approximation in studying the same problem, and gives results similar to those given by Bethe's approximation

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Concerning the scattering of fast electrons and of cosmic-ray particles

By E J WILLIAMS (*Communicated by J Chadwick, F R S—Received 25 October* 1938)

The relativistic theory of nuclear scattering through small angles is discussed, and corrections are applied for the effects of the finite size of the nucleus and the shielding of the nucleus by the atomic electrons This is preliminary to calculations on the multiple scattering of fast electrons by thin metal foils and of cosmic ray particles by metal plates These calculations lead to an accurate estimate of the average theoretical deflexion under the conditions of the experiments, and thereby permit a quantitative interpretation of the observations to be made

The experimental results of Blackett and Wilson on the scattering of cosmic ray particles are in good agreement with the theoretical values, especially if the effect of the finite size of the nucleus is allowed for (The evidence which this agreement provides for the existence of Yukons is discussed) There is no positive evidence in the experiments for a short range non electrical force between the cosmic ray particles and the nuclear particles of the matter traversed, similar to that between slow neutrons and protons It is also inferred that the particles whose scattering was observed produced no nuclear disintegrations in the scattering plates and that the observed anomalous loss of energy by cosmic ray particles with energy around 10^9 V is likely to be due to the production of Yukons

The scattering of fast electrons ($\sim$ 10 mV) by thin lead foils as recently observed by Fowler and Oppenheimer is much less than the present theoretical values This discrepancy seems to fall in line with observations by Klarman and Bothe and by Champion on the single scattering in heavy gases of electrons with energy of the order of 1 mV The scattering of million volt electrons by a thin aluminium foil recently observed by Slawsky and Crane is in good agreement with the present calculations, so that the above discrepancy if real must be associated with a high atomic number There is as yet no indication of how the discrepancy could be accounted for theoretically

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Reproduction in the spotted hyaena *Crocuta crocuta* (Erxleben)

By L H MATTHEWS (*Communicated by A S Parkes, F R S—Received 26 October* 1938)

The external genitalia of the male and female Spotted Hyaena are strikingly similar Over one hundred specimens of the animal were examined in Tanganyika Territory The structure of the genitalia is described, and the changes taking place in them during the sexual cycle are recorded

A reinvestigation of turacin, the copper porphyrin pigment of certain birds belonging to the Musophagidae

By C RIMINGTON, *(Communicated by Sir Henry Dale, F R S —Received 28 October 1938)*

Whilst investigating turacin, prepared from the wing feathers of ***Turacus corythaix***, for possible traces of the III series isomer, it was found that the coproporphyrin obtained by decarboxylation of the uroporphyrin ester appeared to consist almost entirely of coproporphyrin III A thorough examination has therefore been made of the pigment derived from eleven different species of the Musophagidae, which included at least one representative of every turacin bearing genus, except ***Proturacus***

The turacin was extracted from the defatted feathers and converted into the corresponding uroporphyrin ester No attempt was made to obtain a pure uroporphyrin ester at this stage Since the isomeric coproporphyrins are more easily separated than are the uroporphyrins, the entire yield of crude uroporphyrin was decarboxylated in dilute acid under pressure and the resulting coproporphyrin fraction esterified The coproporphyrin methyl ester so obtained was fractionated by a method which serves to separate the series I from the series III isomer In every case crystalline coproporphyrin III methyl ester, with the correct double melting point, was obtained, but in no instance was I successful in isolating or detecting the presence of any coproporphyrin I methyl ester These turacins would appear, therefore to be, substantially, derivatives belonging to series III, to which the normal porphyrin pigments of animals and plants predominantly belong

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The diffusion of radon gas mixtures

By G E HARRISON and W HIRST (*Communicated by M L E Oliphant, F R S —Received 28 October 1938*)

Diffusion measurements have been made for gas mixtures containing radon mixed with hydrogen, helium, neon, argon and air The results of these experiments in the cases of radon hydrogen and radon helium mixtures together with the thermal diffusion experiments already made, suffice to determine the repulsive force between these two pairs of molecules

It is shown that all previous measurements of the coefficient of diffusion of radio active gas mixtures are subject to an error due to the adhesion of the emanation to the walls of the diffusion apparatus which always tends to reduce the experimental values obtained for the diffusion coefficient An experimental study has been made of the means by which adhesion can be made of negligible account in diffusion experiments and an apparatus has been designed which eliminates this disturbing effect The agreement between the final values obtained for the diffusion coefficient of radon gas mixtures is as good as that between the values recently obtained for the coefficient of diffusion of non radioactive gas mixtures

The repulsive force between radon hydrogen and radon helium molecules is compared with that between hydrogen nitrogen, hydrogen carbon dioxide and helium argon molecules The radon molecule is shown to be very "soft"

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6 December, 1938

Observations on the acoustic tubercles or lobi acustico-laterales of bathypelagic fishes and on the functions of the lateral organs By H Muir Evans (*Communicated by E W MacBride, F R S —Received 2 October* 1938)

The directional sensitivity of the retina and the spectral sensitivities of the rods and cones By W S Stiles (*Communicated by Sir John Parsons, F R S —Received* 27 *October* 1938)

A light ray terminating on a given point of the retina has different visual effects depending on its direction of incidence on the retina The direction of incidence can be varied by sending the ray into the eye through different points of the pupil opening The primary object of this work was to determine the effect of direction of incidence on threshold sensitivity, but the results obtained cover the wider problem of the dependence of threshold sensitivity on the condition of stimulation of the retina The test stimulus was a 1° patch of light of wave length λ, exposed for a fraction of a second every few seconds U_λ, the smallest perceptible intensity of the test stimulus, was measured in energy units, the sensitivity then being defined as $1/U_\lambda$ With parafoveal vision of the test stimulus by the dark adapted eye it was found that direction of incidence had little effect on sensitivity when λ was less than 580 mμ, but a pronounced effect for longer wave lengths The dark adapted fovea gave a pronounced directional effect at all wave lengths

The condition of the retina was modified by making the subject view a 10° patch of light (wave length μ and intensity W_μ) at whose centre the test stimulus was applied as an *additional* stimulus For parafoveal vision, the directional effect showed a marked increase in passing from low to high intensities, and the curve connecting the threshold value U_λ and the intensity of the conditioning field W_μ showed a corresponding change of law These two connected effects are attributed to a change from rod vision at low intensities to cone vision at high intensities, the rods and cones being assumed to have different directional properties For foveal vision, which is served by the cones only, somewhat similar effects were observed and were explained by assuming three types of cones for which the relative spectral sensitivities were roughly determined Normally the threshold value increases proportionally with the intensity of the conditioning field at high intensities (Weber's Law) but a striking deviation from this rule was observed for a blue test stimulus on a red conditioning field Increase in the intensity of the conditioning field beyond a certain value produced no corresponding increase in the threshold value This result is ascribed to a special property of the "blue" cones In the present investigation the hypothesis of three types of cone and the determination of their properties have emerged from measurements which are completely independent of colour matching data The connection with the trichromatic system given by colour matching must be close but remains to be elucidated

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The distribution of anthocyanins in flowers, fruits and leaves

By W J C Lawrence, J R Price, (Mrs) G M Robinson and R Robinson, F R S (*Received* 31 *October* 1938)

An analysis is submitted of the results obtained in a wide survey of the distribution of anthocyanins The data are arranged in accordance with a botanical classification and correlation of the chemical findings is attempted on a phylogenetic basis

Assuming an origin from carbohydrate, cyanidin should be the "normal" anthocyanidin since no change in state of oxidation is required in the elaboration of this pigment from two molecules of a hexose and one of a triose This biogenetic theory is supported by the facts that cyanidin derivatives are found to preponderate statistically, that cyanidin derivatives occur in small amount alongside both pelargonidin and delphinidin saccharides, and that autumnal coloration is almost always due to cyanidin derivatives In the latter case we are dealing with a "blind" process having little relation to the more specialized needs of the plant The sequence in the attachment of sugar molecules appears to suggest that the anthocyanidin 3 glycosides are the fundamental types and these give rise by further glycosidation to the 3 biosides and 3 5 dimonosides

In considering the nitrogenous anthocyanins it is noted that Cactales from this point of view should be closely related to the Caryophyllales, Chenopodiales, Lythrales and Thymeleales

Throughout the discussion, species alone are considered but exceptional cases are noted as the result of hybridization

The flexure and torsion of an internally cracked shaft By L A WIGGLESWORTH (*Communicated by G B Jeffery, F R S—Received* 2 *November* 1938)

The Saint Venant theory of flexure and torsion is applied to the bending and twisting of a cracked circular shaft by forces applied to its ends The trace of the crack in the cross section is a slit forming a segment of a diameter of the circle, and is not necessarily central

The treatment of the flexure problem follows a modification introduced by Stevenson of the classical method, and in the only case in which the crack takes effect, which is when the load is transverse to the slit, the problem is to discover three potential functions having certain values on the boundaries of the cross section, one being the classical torsion function

The method of solution is by a conformal transformation of the cross section, followed by the application of an integral formula derived in an earlier part of the paper This formula is the analogue for a region with two boundaries of the complex form of Poisson s integral for a potential function existing inside a circle

The problem is completely solved in finite terms of elliptic functions, and the torsional rigidity, the position of the centre of flexure and the associated twist in the case of flexure are also given in finite terms

Numerical values of these quantities for slits of different lengths are worked out in two cases when the slit is central, and when one end is at the centre of the cross section

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Flexure and torsion of cylinders with cross-sections bounded by orthogonal circular arcs By L A WIGGLESWORTH and A C STEVENSON (*Communicated by G B Jeffery, F R S—Received* 2 *November* 1938)

This paper contains a complete solution of the torsion and flexure functions for elastic beams of cross sections bounded by arcs of two orthogonal circles, i e it includes both circular cylinders with single orthogonal keyways and cylinders of lens shaped cross section The solutions are found by means of conformal transformation

The torsion and flexure moment integrals which relate the constants of the mathematical solution with the external force system applied to the beam are evaluated completely in terms of elementary (circular) functions Although the torsion problem has previously been investigated, this result is new, and the flexure problem has not previously been solved

Expressions are found in finite terms of circular functions for the torsion moment, and also for the associated twist and the coordinates of the centres of flexure and least strain in the flexure problem Numerical computation of these quantities has been carried out and their variations exhibited graphically

Carbohydrate metabolism and muscular exercise

By F C Courtice, C G Douglas, F R S , and J G Priestley (*Received* 2 *November* 1938)

Detailed observations have been made on two human subjects During rest after prolonged muscular work of moderate severity, when there was a persistent low respiratory quotient and ketonuria, the glucose tolerance was found to be reduced, and this reduction appears to be in no way different from the reduced glucose tolerance found without preceding exercise simply as a result of living on a low carbohydrate, high fat diet This supports the conclusion that the main cause of the persistent low quotient after prolonged work is the low ratio of carbohydrate to fat oxidized at that time

The reduced glucose tolerance resulting from a low carbohydrate, high fat diet, as well as that shown after prolonged muscular work, was not accompanied by any significant difference in the response to insulin injected intravenously, and there was therefore no evidence of a decrease in the sensitivity to insulin

In the resting subject intravenous injection of insulin caused, in addition to the characteristic temporary fall of blood sugar, a distinct rise of the respiratory quotient which appeared to signify an increase in the carbohydrate metabolism There was no accumulation of excess lactic acid in the blood, and the effects on the oxygen consumption were slight and inconstant

Subcutaneous injection of adrenaline caused a distinct increase in the oxygen consumption The respiratory quotient showed a quick rise, a succeeding fall to a level below the resting normal, and a final slow recovery, for which a sufficient explanation could be found in the accumulation and disappearance of excess lactic acid which accompanies the hyperglycaemia, without postulating any alteration in the oxidation of carbohydrate

After the simultaneous injection of insulin and adrenaline the changes in blood sugar concentration were diminished, but the changes of lactic acid concentration in the blood and of the respiratory quotient were practically identical with those found with adrenaline alone

An examination of these results makes it clear that though insulin may assist carbohydrate metabolism and adrenaline may help mobilization of liver glycogen there is still much to be done before it will be possible to show in clear detail the precise part that these two endocrine secretions do in fact play in the increased metabolism associated with muscular work

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The absorption of neutrons of medium energy

By J H E Griffiths (*Communicated by F H A Lindemann, F R S —Received* 8 *November* 1938)

The absorption of neutrons produced by the γ rays of radium on Be in eighteen elements has been investigated by comparing the activities of the artificially radioactive isotopes produced The absorption coefficient of the β rays has also been found which enables the relative values of the neutron absorption cross-section to be calculated The results are compared with the theoretical prediction of a smooth increase of cross section with atomic weight and some reasons for the large differences obtained are discussed The absolute value of the cross section is estimated as 1×10^{-25} cm ^{2} for silver compared with the theoretical value of 2×10^{-25} cm ^{2}

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22 DECEMBER, 1938

The nuclear spin of iodine By S TOLANSKY (*Communicated by P M S Blackett, F R S —Received* 3 *November* 1938)

Eight terms in the I^+ spectrum are found to exhibit deviation from the interval rule in their hyperfine structures Those distorted terms with $J = 2$ fail to fit the quadratic deviation formula of Schuler They can be fitted into a cubic interaction formula which is

$$E = A + B \cos IJ + C \cos^2 IJ + D \cos^3 IJ$$

The quadratic term in this expression is attributed to the electrical quadrupole moment of the iodine nucleus The cubic term is shown to be related to the nuclear magnetic moment and may be due to nuclear magnetic octopole moment

A hyperfine structure analysis is given for thirteen newly classified I^+ lines Interval factors are reported for thirty three terms amongst which are five complete sets of multiplets Some of the earlier term identifications require to be modified in the light of recent work

The nuclear spin is proved to be 5/2 without use of the interval rule

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Oxygen evolved by isolated chloroplasts By R HILL (*Communicated by Sir Frederick Hopkins, F R S —Received* 9 *November* 1938)

The observations of earlier workers, that illuminated chloroplasts will produce molecular oxygen apart from the living cell, have been confirmed by an independent method Oxygen was detected and measured spectroscopically by means of haemoglobin Cell free suspensions of chloroplasts were obtained from the leaves of certain angiosperms by grinding in isotonic sucrose solutions After removal from the cells the chloroplasts would only evolve oxygen in light when in the presence of an extract from leaves or in presence of certain ferric salts Carbon dioxide was without influence on this evolution of oxygen, hence the chloroplasts do not show normal assimilation outside the cell even though oxygen may be evolved

In particular the chloroplasts will cause photoreduction of ferric oxalate to ferrous oxalate and oxygen, the oxygen obtained corresponds to the iron reduced The illuminated chloroplasts in the presence of leaf extracts evolved oxygen up to a pressure of 1 mm of mercury, in the presence of ferric oxalate a pressure of 4 mm of mercury can be reached It is concluded that a system can be removed from the cell of a green plant which will convert light energy into a measurable amount of chemical work That molecular oxygen is produced in these circumstances places the system in a category unique among other chemical systems

The activity of this system measured by the initial rate of oxygen production in relation to chlorophyll present is about one tenth of the activity of the living cells under optimum conditions for photosynthesis The results are discussed in relation to the assimilation of carbon by the living plant

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Diffusion of long-chain sulphonic acids By E L McBain, F R S (*Received* 10 *November* 1938)

Diffusion data for the higher soap like long chain sulphonic acids exhibit a sharp minimum in moderately dilute solutions followed by an abrupt rise to double the value in somewhat higher concentrations These measurements seem to demand the presence of both the neutral micelle and ionic micelle found necessary by McBain and collaborators to explain the relation between conductivity and thermodynamic data for colloidal electrolytes

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The influence of pressure on the thermal conductivity By J F Allen and E Ganz (*Communicated by J D Cockcroft, F R S —Received* 21 *November* 1938)

The thermal conductivity of liquid He II has been measured for the liquid under its saturated vapour pressure and under various constant pressures up to twenty atmospheres Over the observed pressure range the conductivity was found to vary linearly with the pressure for all temperatures between 2 03 and 1 17° K The pressure coefficient of thermal conductivity was found to be negative between 2 03 and 1 63° K and positive below 1 63° K The positive value below 1 63° K was explained by the increased density and specific heat of compressed He II Above 1 63° K, on the other hand, the increased hindrance to the motion of the heat carrying atoms when the liquid is compressed, was considered to outweigh the increase in density and specific heat

By means of the dimensional equation of thermal conductivity, a value was calculated for the product of velocity and mean free path of the heat carrying mechanism The variation of this value with temperature bears a close resemblance to the variation of velocity with temperature both for the flow of liquid He II through very fine capillaries and for the flow of mobile surface films of He II

Although the apparatus was not designed to measure the conductivity of a poorly conducting substance, an upper limit to the thermal conductivity of solid helium can be given as 1/1000 of that of liquid He II

The action of radiations on bacteria IV The lethal action of soft X-rays

By R B Haines and D E Lea (*Communicated by Sir Joseph Barcroft, F R S —Received* 23 *November* 1938)

An account is given of the experimental methods and the results obtained in a research on the lethal action of soft X rays (wave lengths 8 32, 4 15 and 1 54 Å) on a number of different bacteria The survival curves are exponential and the mean lethal doses are independent of intensity For the production of these radiations a tube is used in which the target serves also as the window, and with which very high intensities can be economically obtained The measurement of the intensity and homogeneity of the radiation is also described

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The action of radiations on bacteria V The target theory

By R B Haines and D E Lea (*Communicated by Sir Joseph Barcroft, F R S — Received* 23 *November* 1938)

On the basis of the target theory an attempt is made to give a detailed explanation of the variation of mean lethal dose with the ionization density of the radiations The experimental data are the mean lethal doses of a number of vegetative bacteria and spores exposed to α , β and γ rays and to soft X rays of wave lengths 8 32, 4 15 and 1 54 A, the ionization density of these radiations in biological material varying from 10^5 to 4×10^7 ionizations per cm of track The vegetative cells show a gradual increase of mean lethal dose with ionization density The spores on the other hand show independence of lethal dose on ionization density except at the highest density (α particles) when the mean lethal dose is only a quarter as great as for X and γ rays

In attempting to interpret these results in terms of the target theory, it is assumed that the organism contains a single spherical target of radius 10^{-5} cm , in which several ionizations must be produced simultaneously to cause death, the required number being about 5 for vegetative bacteria and about 40 for spores Agreement is satisfactory in the case of vegetative cells, but less good with spores

An alternative theory is that a bacterium contains about a thousand targets each of about 5×10^{-7} cm radius, the inactivation of a single target sufficing to render the cell non viable On this theory in vegetative cells the targets are single molecules and a single ionization or ion cluster suffices to inactivate the target, in the case of spores the targets contain their molecules in duplicate and both molecules must be affected to render the cell non viable The lethal doses of various radiations predicted by this theory are in good agreement with experiment

The possibility is discussed that these targets have something of the significance in bacteria of genes in higher cells and that loss of viability by radiation is the result of "lethal mutation"

Theory of the fatigue of metals By E Orowan, *Birmingham*

(*Communicated by M L E Oliphant, F R S —Received* 23 *November* 1938)

Owing to the microscopic inhomogeneity of the stress distribution, a plastic metal will yield at the local stress peaks when the bulk of the material is still elastic, and the fatigue of the metal is caused by the phenomena taking place in these microscopic plastic regions The decisive point is that the plastic spots are not directly connected with the external load, the load gives first, a certain elastic deformation of the bulk of the specimen, and the stress acting in the plastic spots depends on the plastic strain they suffer If they are very soft, the plastic strain in them is very large and the stress very small, and vice versa For a given external load, applied to the specimen as a whole, the stress in a plastic spot is a linear function of its plastic strain, decreasing with increasing strain From this linear "stress characteristic" of the plastic spot and from its strain hardening curve (for a given rate of loading) its plastic deformation in the course of the load cycles can be derived graphically or analytically The result is that the amplitude of the plastic strain decreases in the course of the load cycles, owing to the increase of stress caused by strain hardening, it tends towards zero as the terms of a geometric series, the total plastic strain (the sum of the absolute amounts of all positive and negative strains) being always finite If the total plastic strain is smaller than the critical strain the material can endure without fracture, then the range is safe, otherwise unsafe

The theory developed on these lines gives, beside the explanation of the existence of safe and unsafe ranges, log S log N curves which are in full accord with the typical curves observed It explains the fact that there is a correlation between strength and fatigue endurance, but no correlation between plastic properties and fatigue endurance According to the theory, the only general statement that can be made on the relation between the limiting safe range and the mean stress is that, below the elastic limit, the limiting safe range is nearly independent of the mean stress—a rule deduced by McAdam from empirical data Conclusions from the theory about the influence of the cross sectional area and of temperature upon fatigue, and about properties of mechanical hysteresis, are in accord with observations

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Magnetic susceptibilities and related properties of rare earth crystals I

By G J KYNOH and W G PENNEY (*Communicated by S Chapman, F R S —Received* 28 *November* 1938)

This paper shows that it is impossible to reconcile current interpretations of the absorption spectra and specific heat measurements on hydrated rare earth crystals with the magnetic properties The investigation proceeds by developing the magnetic susceptibility tensor as a power series in the parameters of the crystalline potential acting on the magnetic ion, the predominant part of which has cubic symmetry The mean, or powder, susceptibility is independent to the first order of the non cubic part of the potential In spite of this fact, the agreement between the observed values of the mean magnetic susceptibility and the theoretical values based on the accepted spectroscopic energy levels is poor Further, to account for the magnetic anisotropy of the crystals, splittings of at least 20 cm $^{-1}$ must be present in most states which are degenerate in the pure cubic potential field Such splittings should be plainly visible in the absorption spectra, but they are not found

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Metabolism and morphogenesis, studies with the cartesian diver ultra-micro-manometer I Anaerobic glycolysis of the regions of the amphibian gastrula

By E J BOELL, J NEEDHAM and V ROGERS (*Communicated by Sir Frederick Hopkins, F R S —Received* 28 *November* 1938)

Gastrulation is a stage of crucial importance in embryonic development, for the nerve tube, the main vertebrate axis, is then called into being by a stimulus exerted from the invaginating archenteron roof An understanding of how the active substance is liberated in the dorsal lip of the blastopore and not in other regions, such as ventral ectoderm, necessitates a more precise knowledge of the metabolic properties of the various regions of the gastrula, but hitherto the smallness of the material has precluded such studies

The application of the cartesian diver as a micro manometer suitable for this work is now described Its sensitivity may be gauged from the fact that whereas 1 cm on the standard Warburg manometer scale corresponds to a gas change of 20 cu mm , 1 cm on the diver manometer scale corresponds to a gas change of about 0 008 cu mm A full account of the calibration and use of the instrument is given, together with a number of new techniques such as the employment of strong lithium chloride solution as the flotation medium for the divers, which permits of ammonia estimations and Kjeldahl determinations on their contents For the experiments with regions of the gastrula, pieces of tissue containing between 5 and 15γ Kjeldahl nitrogen were used

The main series of results, which could be placed on a statistical basis, showed that the anaerobic glycolysis and the anaerobic ammonia production of the dorsal lip of the blastopore were three times as high as those of the ventral ectoderm In these respects, therefore, the metabolism of that part of the gastrula where the organiser substance (evocator) is liberated was considerably more intense than that of the ventral ectoderm, where the substance is never liberated in normal development

Metabolism and morphogenesis, studies with the cartesian diver ultra-micro-manometer II Effect of dinitro-o-cresol on the anaerobic glycolysis of the regions of the amphibian gastrula

By E J BOELL and J NEEDHAM (*Communicated by Sir Frederick Hopkins, F R S —Received 28 November* 1938)

In view of the effects which nitrophenols have been found to have on various aspects of fermentation and respiration, it was thought interesting to examine the action of this substance on the anaerobic glycolysis of the dorsal lip region and the ventral ectoderm of the amphibian gastrula It resulted that the anaerobic glycolysis of the dorsal lip was very little affected, but that of the ventral ectoderm was raised to approach more nearly the former This raising of activity of ventral ectoderm to approximate more nearly to dorsal lip activity was due almost entirely to that part of the carbon dioxide production uncompensated for by ammonia production, since the latter was unaffected

A comparison is suggested between the two regions of the gastrula on the one hand, and the metabolism of unfertilized as compared with fertilized echinoderm eggs, or the metabolism of actively developing as compared with diapause embryos of the grasshopper, on the other In all three cases, the less active metabolism (amphibian gastrula ventral ectoderm, unfertilized echinoderm egg, diapause orthopteran embryo) seems to be in some way physiologically "damped", since by added substances such as reversibly reducible dyes, nitrophenols, etc it can be raised almost to the level of the most active parts or stages, while these themselves cannot be raised by the addition of special substances Such damping appears to play a very important part in morphogenesis and will merit much further examination

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Metabolism and morphogenesis, studies with the cartesian diver ultra-micro-manometer III Respiratory rate of the regions of the amphibian gastrula

By E J BOELL and J NEEDHAM (*Communicated by Sir Frederick Hopkins, F R S —Received* 28 *November* 1938)

The adaptation of the diver manometer method for the measurement of oxygen consumption is described, together with appropriate tests of its reliability

Applied in two thorough series of experiments to the dorsal lip region and the ventral ectoderm of the amphibian gastrula, it showed that no difference whatever was perceptible between the oxygen consumption of the two regions Taken in conjunction with the results of the first paper of the series on anaerobic glycolysis this requires the conclusion that the change which occurs at the blastopore when invagination begins is a change in quality of metabolism rather than one of quantity

A smaller number of estimations of oxygen consumption carried out on the roof of the blastula and on the closing neural folds also failed to show any significant differences between them although indications had been obtained in the first paper of the series that their ammonia production is significantly different

These results appear to conflict with those recently published by other authors in which the oxygen consumption of the two hemispheres of intact amphibian gastrulae was measured, but the difference is one of interpretation rather than of observation That the hemisphere which contains the dorsal lip shows a higher respiratory rate than that which contains the ventral ectoderm is no doubt true, but the gastrula is a relatively complicated morphological structure, and it is extremely probable that the dorsal lip hemisphere contains more highly respiring tissue than the other hemisphere

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Metabolism and morphogenesis, studies with the cartesian diver ultra-micro-manometer IV Respiratory quotient of the regions of the amphibian gastrula By E J Boell, H Koch and J Needham (*Communicated by Sir Frederick Hopkins, F R S —Received* 28 *November* 1938)

The adaptation of the diver manometer method for the measurement of respiratory quotient is described, together with appropriate tests of its reliability It necessitates the suspension of alkali and acid drops in the waxed neck of the diver, and these are blown down into the bulb, when the oxygen consumption has proceeded sufficiently far, by an excess pressure of about 12 in mercury, thus liberating the carbon dioxide As the oil drop returns exactly to its original position when the pressure is released, the carbon dioxide can be correctly measured and the R Q obtained

When the regions of the amphibian gastrula were studied with this method, it was found that both dorsal lip region and ventral ectoderm show a trend towards R Q 1 0 during gastrulation, but that the former reaches it almost as soon as the process has begun, while the latter has only attained an R Q of about 0 85 by the time it is underlain by invaginated mesoderm

This result is in general agreement with those of the preceding papers in the series in which it was shown that the anaerobic glycolysis but not the respiratory rate of the dorsal lip region is increased during gastrulation It is also in general agreement with the loss of glycogen from the roof of the archenteron demonstrated by previous authors to be localized to that part of the gastrula Lastly, it is consonant with the view, developed on quite other grounds, that the primary organizer substance (evocator) exists in masked form in combination with glycogen and protein, and is liberated in active form only in the dorsal lip region during normal development

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Some problems of localization in the central nervous system (Ferrier Lecture) By E D Adrian, F R S (*Received* 30 *November* 1938)

By electrical stimulation of the brain, David Ferrier established the main facts relating to the localization of the motor centres in the cerebral cortex These facts have been confirmed, on man as well as on animals, and have been of great practical as well as theoretical importance But although they have been the basis for all attempts to analyse the cerebral organization, they reveal mainly its receiving and executive apparatus and show little of the intervening processes With the development of methods for recording the activity of the brain in terms of the electrical effects which it produces, the problems of cerebral localization can be approached from another aspect It has been found that the cerebral cortex is naturally in a state of constant activity which may take the form of a rhythmic series of potential waves (e g Berger's α rhythm) or of more complex and irregular oscillations When the activity has been reduced by an anaesthetic the arrival of an afferent message has a twofold effect It produces a direct local excitation in the region in which it arrives and a more widespread increase in the persistent activity of this and neighbouring regions The latter result may be considered a local or regional awakening, for it resembles the generalized increase in electrical activity which occurs in the brain when a sleeper is roused In either case, a mutual reinforcement by cortex and thalamus is an important factor in raising and maintaining the level of the "spontaneous" activity

In certain cases electrical stimulation of the brain has the same facilitating effect as an afferent message The spread of activity can then be followed by making simultaneous records of the potential changes at different distances from the stimulated point, and it can be shown how a number of competing wave systems can come into being

Electrical records have not yet revealed any features peculiar to the cerebral nerones In other parts of the nervous system there are many examples of rhythmic activity favoured by the arrival of impulses, and in the cortex, as elsewhere, there are probably all grades of spontaneity, ranging from nerone systems which cannot help discharging periodically to those which need a specific stimulus for each reaction Thus, the activity in a given region of the cerebrum will depend on the interactions of the rhythmic and non rhythmic systems In normal conditions inhibition as well as facilitation will help to determine the local patterns of activity, but in records from the narcotized brain the excitatory factors are the more prominent

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The role of blue-green algae in nitrogen fixation in rice-fields

By P K De (*Communicated by F E Fritsch, F R S —Received 30 November 1938*)

Since it has been established that abundant nitrogen fixation takes place in the soils of Indian rice fields, as well as in cultures of such soils in the laboratory in which blue green algae are nearly always present in quantity, the possible role of such blue green algae in nitrogen fixation has been investigated with positive results Unialgal cultures of six different species were readily obtained, but the removal of the associated bacteria proved to be a much more difficult task, and it was only after many failures that four species were obtained in a completely pure condition, devoid of all foreign organisms This was ultimately accomplished by a process of repeated subculturing on sterilized silica gel plates according to a method first worked out by Pringsheim

Inoculations from these pure cultures, grown in mineral solutions lacking nitrogen (except for that contained in small traces of soil extract), in part under constant light and temperature conditions, showed that the three species of *Anabaena* which had been isolated were all able to fix considerable quantities of nitrogen, averaging 3–5 mg /100 c c of medium in about two months On the other hand, a fourth member of the blue green algae, *Phormidium foveolarum*, afforded no evidence at all of any capacity for nitrogen fixation The amount of nitrogen fixed by the species of *Anabaena* in the presence of *Azotobacter* and other bacteria is about the same as in the pure cultures containing the alga only, from which it appears that in the presence of algae bacteria play a relatively unimportant role in nitrogen fixation in these soils A considerable part of the nitrogen fixed is found in the external medium

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The submicroscopic structure of dental enamel By J THEWLIS (*Communicated by W L Bragg, F R S —Received* 30 *November* 1938)

From observations made on photomicrographs and X ray diffraction photographs of human deciduous enamel, the submicroscopic structure of such enamel has been studied

It has been found that each enamel prism is composed of a multitude of crystallites of apatite which may be divided into two groups, so arranged that their hexagonal axes tend to make definite angles with the direction of the prism The values of these angles are approximately 5 and 40° and the crystallite axes are inclined so as to point to that side of the prism remote from the nearest cusp, the directions being taken away from the tooth

In general both the above groups of crystallites are present in the prism, with the 5° group predominant, although occasionally both groups are equally represented Sometimes, however, the 40° group is entirely absent In some cases variations in the degree of perfection of the crystalline orientation within individual prisms may occur, thereby giving rise to the three types of X ray photograph observed In other cases these different types of photograph may be due to variations in prism regularity

The interprismatic substance, like the prism material, usually contains the 5 and 40° groups of apatite crystallites but nothing can be said as regards the variation in degree of perfection of the crystalline orientation

The submicroscopic structure of enamel in certain cases has been discussed and illustrated diagrammatically

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A theory of radiation By G A SCHOTT, F R S , prepared for publication by T LEWIS (*Communicated by A W Conway, F R S —Received* 30 *November* 1938)

The paper attempts to account for the observed properties of radiation on classical lines, in particular, the line character of spectra, the Planck Einstein law, the possibility of interference over long distances and the capability of ionizing atoms or molecules

Structure and thermal properties associated with some hydrogen bonds in crystals Part I The isotope effect

By J MONTEATH ROBERTSON and A R UBBELOHDE (*Communicated by Sir William Bragg, P R S —Received 2 December* 1938)

The hydrogen deuterium isotope effect has been investigated for a number of carboxyl and hydroxyl groups in crystals, in order to obtain fresh information on the hydrogen bond, and the possibility of intermolecular resonance X ray methods have been developed for the accurate measurement of the changes in crystal spacing on substituting deuterium, and in the case of oxalic acid dihydrate the complete section of the deformation ellipsoid has been calculated in relation to the crystal structure

In all cases an expansion is observed on substituting deuterium, the observed effect being largest for oxalic acid dihydrate, and practically zero for phthalic acid and β resorcinol Intermediate expansions have been observed for $NaHCO_3$, succinic and benzoic acids, and α resorcinol For $(COOH)_2 2H_2O$ the expansion is markedly directional, and the observed effect can most simply be explained by assuming an expansion of the short hydrogen bond, and a contraction of the longer hydroxyl bonds in this crystal A tentative interpretation of this effect is discussed in terms of resonance between the energy levels of two alternative molecular structures in the crystal

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Heterogeneous catalysis and enzyme reactions Part I The kinetics of heterogeneous catalysis Part II Enzyme systems

By E C C BALY, F R S (*Received* 2 *December* 1938)

When a substance is adsorbed on a surface, adsorption complexes are formed in which an adsorbing unit in the surface donates one or more rotation vibration quanta to each molecule of the adsorbate If this donated energy be Q calories, the supply of Q calories will set free the activated molecules of the adsorbate which can then undergo or take part in a reaction, the energy of activation of which is Q calories The reaction velocity of all heterogeneously catalysed reactions is expressed by the following equations

$$r = \frac{k_1 R S n k_2 e^{-nQ/RT}}{k_1 R + n k_2 e^{-nQ/RT}},$$

$$\frac{1}{r} = \frac{1}{S n k_2 e^{-nQ/RT}} + \frac{1}{k_1 RS},$$

and

$$\log \frac{r}{k_1 R S^{-r}} = \log \frac{n k_2}{k_1 R} - nQ'/T,$$

where S is the number of adsorbing units in the surface, R is the concentration of the reactant, Q is the total energy of activation of the reaction, n is the reciprocal of the number of components of the catalyst, Q' is $Q/4\ 5787$ and k_1 and k_2 are constants

Enzymes are two-component catalysts which are formed by the action of the "activator" on the precursor and the mechanism of this action is discussed

It is shown that all heterogeneous catalysts can promote reactions, the energies of activation of which are submultiples of the maximum energy which they can donate to the reactant In the case of two component catalysts, such as enzymes, there is an integral relation between the maximum energy they can donate and their energy of deactivation

With substances of large molecular weight there must exist a close correlation between the structure of the catalyst and of the reactant, and in this is to be found the explanation of the specificity of all enzymes

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The photosynthesis of carbohydrates from hydrated carbon dioxide

By E C C BALY F R S (*Received* 2 *December* 1938)

The photosynthesis of carbohydrates from hydrated carbon dioxide adsorbed on the surface of nickel oxide is achieved by two consecutive light reactions

$$(1)\quad 4\mathrm{NiO},\ \mathrm{CO_2H_2O} + h\nu_1 = 2\mathrm{Ni_2O_3},\ \mathrm{CHOH},$$

$$(2)\quad 2\mathrm{Ni_2O_3},\ \mathrm{CHOH} + h\nu_2 = 2\mathrm{Ni_2O_3} + \mathrm{CHOH},$$

where $h\nu_1$ is a quantum of blue light ($\lambda = 4000$ A), $h\nu_2$ is a quantum of red light $\lambda = 6566$ A) and CHOH is an activated molecule of formaldehyde, six of which undergo cyclic polymerization into a molecule of a hexose

These two light reactions are followed by a thermal reaction

$$(3)\quad 2\mathrm{Ni_2O_3} = 4\mathrm{NiO} + \mathrm{O_2},$$

which renders the photosynthesis a continuous process

A large surface of NiO is unsuited for photosynthesis, since one group of 4Ni and 4O atoms out of seven such groups is redundant In a crystal lattice of NiO containing $\mathrm{ThO_2}$ in the molecular ratio of $1\mathrm{ThO_2}$ 24NiO, each $\mathrm{ThO_2}$ molecule replaces one group of 4Ni and 4O atoms out of every seven such groups and thus renders the surface ideal for photosynthesis

When such a surface is irradiated in the presence of hydrated carbon dioxide, there is photosynthesized an unstable complex carbohydrate which changes to a type of starch When pure NiO containing very small crystal units is irradiated in presence of hydrated carbon dioxide, hexoses only are photosynthesized

A very close analogy between photosynthesis *in vitro* and photosynthesis *in vivo* has been established The explanation has also been found of the fact that in both processes the rate of photosynthesis increases with the temperature up to a definite limit and when the temperature exceeds that limit the rate of photosynthesis rapidly decreases, the limiting temperature depending on the intensity of the light

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The adsorption of the hydrous metallic oxides by kieselguhr

By E C C Baly, F R S, W P Pepper and C E Vernon (*Received 2 December* 1938)

The adsorption of the oxides of aluminium, thorium, ferric iron, cobalt and nickel on specially purified kieselguhr has been investigated and the relation between the amount of each oxide adsorbed on 100 g of the kieselguhr and the ζ potential in 0 05 N acetic acid of the coated kieselguhr was determined in each case It was found that the ζ potential, which in each case was positive, had a maximum value when the kieselguhr was completely coated with the oxide and that this maximum value was materially greater than that of the unsupported oxide

The adsorption of aluminium and thorium oxides is restricted to one unimolecular layer, and in the case of ferric, cobaltous and nickelous oxides the adsorbate consists of three uni molecular layers When cobalt oxide or nickel oxide containing thorium oxide in the molecular ratio of $1ThO_2$ to 24CoO or 24NiO is adsorbed there is formed a single layer of crystal unit cells, in each of the three layers of which one group of 4Ni or 4Co atoms and 4O atoms out of every seven such groups is replaced by $1ThO_2$ molecule

The effective surface area of 100 g of the kieselguhr was found to be 2 86893 × 10^6 sq cm The diameter of the molecules of aluminium oxide, ferric oxides and thorium oxide in the unimolecular adsorbed layer were found to be 6 242 × 10^{-8} cm, 9 10 × 10^{-8} cm and 5 72 × 10^{-8} cm, respectively

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Studies in the viscosity of colloids I

By J R Robinson (*Communicated by E K Rideal, F R S—Received* 5 *December* 1938)

The nature of the anomalous viscosity of dilute suspensions of rigid, anisometric, colloidal particles has been elucidated by a combination of simultaneous measurements of the optical and viscous behaviour of the suspensions with an approximate theoretical treatment

It is found that with increasing rates of shear, the particles become more and more orientated, the orientation being measured in direction by the angle of isocline, and in intensity by the double refraction of flow Meanwhile the over all viscosity falls towards that of the solvent

It is shown that the overall viscosity exceeds that of the solvent as a result of energy dissipated through the disturbance of the field of flow of the solvent by the precessional motions which it imposes upon the particles As increasing rates of shear are applied, these precessional motions change in a manner which, statistically considered, constitutes the orientation of the particles In the course of this orientation, the extra dissipation of energy due to the particles becomes less in comparison with that involved in the flow of the solvent alone This is the physical basis of the anomalous viscosity of the systems studied

On fluctuation in electromagnetic radiation. By M Born and K Fuchs

(*Communicated by E T Whittaker, F R S —Received 5 December 1938*)

It is shown that all previous treatments of the fluctuations of radiation are inadequate One cannot expect a complete coincidence of the formulae derived from statistical mechanics and from wave theory, as the zero energy plays a different role in these methods The wave fluctuations are here recalculated for a quantized electromagnetic field, and it is shown that the result coincides with the statistical formula as far as can be expected, provided one overlooks the divergence of the zero energy The importance of this infinity is discussed with regard to the question of the limitations and future improvement of the present radiation theory

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On the formation and structure of multilayers By J J Bikerman

(*Communicated by E K Rideal, F R S —Received 6 December 1938*)

The "built up" multilayers of Langmuir and Blodgett are shown to consist of films stretched between the irregularities of the solid surface and touching the surface only at discrete points This view is based on measurements of the "deposition ratio" during the transfer of monomolecular films from water onto grooved surfaces and wire gauzes

A technique of obtaining multilayers on wire gauzes is described The behaviour of these multilayers is investigated and on their analogy it seems probable and in some cases certain, that the stretched films which are the first product of "building up" multilayers burst soon after their preparation and give rise to gel structures or more or less oriented micro crystals

An explanation is advanced for the various kinds of deposition (X Y and Z films) The hydrophilic character of the surface of the films increases in the order $X < Y < Z$, the type of deposition is determined by the wettability of the surface, and is related to the contact angles

As the measure of the wettability the frictional force between a water drop and a multilayer is employed In disagreement with Coulomb's (or Amonton's) law this frictional force is almost independent of the weight of drop A qualitative interpretation of this behaviour is given

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The nature of atmospherics VI By F E Lutkin

(*Communicated by Sir George Simpson, F R S —Received 7 December* 1938)

The study of atmospherics originating from British storms indicates that 80 % of the lightning flashes occurring in England destroy negative cloud moments In storms of the heat type destruction of negative cloud moments account for almost all the flashes, while in storms of the "frontal" type positive moments are destroyed as frequently as negative ones This is accomplished in most cases by a sequence of discharges four or five in number, probably down the same ionized channel, occurring in about $\frac{1}{10}$ sec Two thirds of these discharges themselves consist of a sequence of three or four current pulses in the lightning channel occurring in about $\frac{1}{1000}$ sec In the remaining third, the discharge consists of twenty pulses, and this type, often preceding the main stroke, is thought to act as leader and establish the ionized channel Further work with two or more stations within 20 km of the source is necessary to establish definitely whether this negative moment is in all cases destroyed by discharges to earth Of the remaining 20 % of the flashes, many, although indicating an initial destruction of positive moment, show finally the destruction of negative moment

The average peak current value appears to be about 30,000 amp while the average charge dissipated by each current pulse is about $\frac{1}{3}$ coulomb Maximum values of 200,000 amp have been registered

The multiplicity of current pulses produce oscillatory wave trains of a few or many cycles depending on the nature of the discharge Each wave train consists of a series of field changes with maximum amplitude up to 600 V/m , according to the distance from the source Single oscillations last less than a millisecond while groups may last for $\frac{1}{10}$ sec or more

Similar oscillatory field changes arise from more distant storms, those from distances of 3000 km reaching 0 5 V/m From sources over sea the duration of the individual oscillatory trains is greater than in the case of those originating over land

While the predominant frequency of these oscillations is of the order of 10 kc /sec reflexions from low ionized regions are thought to produce the high frequency embroidery seen on patterns from distances of 10–100 km and result in such atmospherics being troublesome in the medium wave bands

The specific heat of gases at high temperatures By R W Fenning and A C Whiffin (*Communicated by W L Bragg, F R S —Received 7 December 1938*)

The information that was required on the compressibility characteristics of gas mixtures at medium pressures, in connexion with a redetermination of the specific heat of gases at high temperatures, led to this quantity being obtained experimentally The paper gives a description of the apparatus that was used for the purpose and particulars of its calibration It also gives examples of the departure of certain gas mixtures from the ideal state both at 20 and 100° C The apparatus is regarded as being suitable for work of high precision provided it is used in conjunction with sufficiently sensitive instruments for measuring temperature and pressure

The principal magnetic susceptibilities of neodymium sulphate octahydrate at low temperatures By L C Jackson (*Communicated by A M Tyndall, F R S —Received 7 December* 1938.)

The principal magnetic susceptibilities of the crystals of neodymium sulphate octahydrate have been determined over the temperature range 290–14° K All three susceptibilities obey the law $\chi(T+\Delta) = \text{const}$ above about 120° K, the three curves on the $1/\chi$ T graph being parallel straight lines At low temperatures one of the principal susceptibilities tends to become independent of temperature while the other two increase more rapidly with decrease in temperature below 60° K than they do at higher temperatures The magnetic ellipsoid rotates through a considerable angle as the temperature decreases from 290 to 14° K At room temperature the anisotropy of the crystal ($\Delta\chi/\chi$) is 11% but this increases to 70% at 14° K The bearing of these results on the crystalline field theory is referred to in the following paper by Dr W G Penney

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Spiral structure and chromosome pairing in *Osmunda regalis*. By I Manton (*Communicated by W H Lang, F R S —Received* 8 *December* 1938)

The paper contains a study of the last three sporangial divisions in *Osmunda regalis* by a variety of technical methods including the ammonia method for spiral structure The study of the premeiotic division is amplified by observations on mitoses in the tapetum Evidence is given on the number of coils in a chromosome at mitosis and at meiosis, on the length of the chromosome thread, whether spiral or otherwise at a number of significant stages and on chromosome elasticity at all three types of division The existence of long range elasticity beyond what would be expected from the spiral configuration alone is suggested both at mitosis and at meiosis, the evidence for it at anaphase of the first meiotic division being complete The chromosome length appears to be relatively constant throughout the mitotic cycle but chromosome pairing appears to be preceded by a real elongation of at least 50% and to be followed by a real shrinkage also of the order of 50% An interpretation of these facts is given in terms of the known molecular structure of fibrous proteins

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